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* * * * * STN Columbus * * * * *
FILE 'HOME' ENTERED AT 12:24:44 ON 31 MAY 2005
=> file biosis caba caplus embase japio lifesci medline scisearch uspatfull
=> e frey joachim/au
E1      1      FREY JERGEN/AU
E2      1      FREY JIM/AU
E3      298 --> FREY JOACHIM/AU
E4      3      FREY JOANA/AU
E5      1      FREY JOCHEN/AU
E6      1      FREY JOERG/AU
E7      1      FREY JOHANN H/AU
E8      16     FREY JOHANN R/AU
E9      35     FREY JOHANN RUDOLF/AU
E10     10     FREY JOHANN WILHELM/AU
E11     4      FREY JOHN/AU
E12     1      FREY JOHN A/AU
=> s e3 and salmonicida
L1      17 "FREY JOACHIM"/AU AND SALMONICIDA
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2      8 DUP REM L1 (9 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 8 ANSWERS - CONTINUE? Y/(N):y

L2      ANSWER 1 OF 8  USPATFULL on STN
AN      2005:68524  USPATFULL
TI      Novel type III secretion pathway in Aeromonas ***salmonicida*** , and
        uses therefor
IN      ***Frey, Joachim*** , Bern, SWITZERLAND
        Stuber, Katja, Ittigen, SWITZERLAND
        Thornton, Julian C., Victoria, CANADA
        Kuzyk, Michael A., Richmond, CANADA
        Burian, Jan, Victoria, CANADA
PA      Universitat Bern (non-U.S. corporation)
PI      US 2005058662      A1  20050317
AI      US 2004-813908      A1  20040326 (10)
RLI     Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.
        No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN
PRAI    US 2000-248864P      20001115 (60)
DT      Utility
FS      APPLICATION
LREP    KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,
        97204
CLMN    Number of Claims: 15
ECL     Exemplary Claim: CLM-01-22
DRWN    5 Drawing Page(s)
LN.CNT  1427
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      Disclosed is a newly identified and characterized type III secretion
        system in Aeromonas ***salmonicida*** . The invention also
        encompasses the use of components of the novel secretion system in
        immunoprotection against A. ***salmonicida*** infection, as well as
        other diagnostic and therapeutic uses thereof.

L2      ANSWER 2 OF 8  BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
        DUPLICATE 1
AN      2003:569435  BIOSIS
DN      PREV200300562558
TI      The ADP-ribosylating toxin, AexT, from Aeromonas ***salmonicida***
        subsp. ***salmonicida*** is translocated via a type III secretion
        pathway.
AU      Burr, Sarah E.; Stuber, Katja; ***Frey, Joachim*** [Reprint Author]
CS      Institute of Veterinary Bacteriology, University of Berne,
        Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland
        joachim.frey@vbi.unibe.ch
SO      Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591.
        print.
        CODEN: JOBAAY. ISSN: 0021-9193.
DT      Article
LA      English
ED      Entered STN: 3 Dec 2003

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Last Updated on STN: 3 Dec 2003

AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen *Aeromonas salmonicida* subsp. *salmonicida*. The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have identified a further 12 open reading frames that possess high homology to genes encoding both structural and regulatory components of the *Yersinia* type III secretion apparatus. Using marker replacement mutagenesis of *aopB*, the *A. salmonicida* subsp. *salmonicida* homologue of *yopB* in *Yersinia*, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An *acrV* mutant of *A. salmonicida* subsp. *salmonicida* displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of  $\text{CaCl}_2$  concentrations as high as 10 mM. This *acrV* mutant is also unable to translocate AexT into the cytosol of fish cells, indicating *AcrV* is involved in the translocation process. Inactivation of either the *aopB* or *acrV* gene in *A. salmonicida* subsp. *salmonicida* (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant *AcrV*-His protein provides fish cells protection against the toxic effects of the bacterium.

L2 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2

AN 2003:720743 CAPLUS

DN 139:359791

TI Type III secretion genes in *Aeromonas salmonicida* subsp. *salmonicida* are located on a large thermolabile virulence plasmid

AU Stuber, Katja; Burr, Sarah E.; Braun, Martin; Wahli, Thomas; Frey, Joachim

CS Institute of Veterinary Bacteriology, University of Bern, Bern, CH-3001, Switz.

SO Journal of Clinical Microbiology (2003), 41(8), 3854-3856  
CODEN: JCMIDW; ISSN: 0095-1137

PB American Society for Microbiology

DT Journal

LA English

AB Type III secretion genes in *Aeromonas salmonicida* subsp. *salmonicida* are located on a large plasmid of approx. 140 kb, termed pASvirA. Cultivation of this organism at elevated temps. such as 25.degree.C can, however, result in loss of this plasmid. This is accompanied by an inability to secrete toxin AexTa and by a loss of virulence for cultured fish cells.

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 3

AN 2004:164565 BIOSIS

DN PREV200400168104

TI Association of Type III secretion genes with virulence of *Aeromonas salmonicida* subsp. *salmonicida*

AU Burr, Sarah E.; Wahli, Thomas; Segner, Helmut; Pugovkin, Dmitri; Frey, Joachim [Reprint Author]

CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, 3012, Berne, Switzerland  
joachim.frey@vbi.unibe.ch

SO Diseases of Aquatic Organisms, (December 3 2003) Vol. 57, No. 1-2, pp. 167-171. print.  
CODEN: DAOREO. ISSN: 0177-5103.

DT Article

LA English

ED Entered STN: 24 Mar 2004

Last Updated on STN: 24 Mar 2004

AB *Aeromonas salmonicida* subsp. *salmonicida* possesses a number of potential virulence factors, including a recently identified plasmid-encoded Type III secretion system. A number of field isolates of *A. salmonicida* subsp. *salmonicida* were examined for the presence of Type III secretion genes. Using in vitro experiments, it was found that field isolates containing such genes are cytotoxic to fish

cell lines, whereas those that lack these genes are not. Using a rainbow trout in vivo model, the virulence of a wild type A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strain (Strain JF2267), which possesses Type III secretion genes, was compared to that of a laboratory derivative of the same strain that has lost these genes. While Strain JF2267 was virulent towards rainbow trout, its derivative was not. The A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* Type Strain ATCC 33658T, which also lacks Type III secretion genes, was also found to be avirulent by this challenge model. The findings from both the in vitro and in vivo experiments suggest that the presence of Type III secretion genes is associated with the virulence of this important fish pathogen.

L2 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391747 CAPLUS

DN 136:385049

TI Novel exoenzyme toxin of Aeromonas \*\*\*salmonicida\*\*\* and use for recombinant salmon furunculosis vaccine

IN \*\*\*Frey, Joachim\*\*\* ; Kuhnert, Peter; Braun, Martin; Thornton, Julian C.; Kuzik, Michael A.; Burian, Jan

PA Switz.

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 2002023328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		

AB A novel protein toxin named Aeromonas \*\*\*salmonicida\*\*\* exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of A. \*\*\*salmonicida\*\*\*. Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against A. \*\*\*salmonicida\*\*\* infections.

L2 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391746 CAPLUS

DN 136:400588

TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines

IN \*\*\*Frey, Joachim\*\*\* ; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; Burian, Jan

PA Switz.

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,			

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,  
 UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,  
 CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,  
 BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses the use of components of the novel secretion system in immunoprotection against *A. \*\*\*salmonicida\*\*\** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of *Aeromonas \*\*\*salmonicida\*\*\** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L2 ANSWER 7 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 4

AN 2002:564959 BIOSIS

DN PREV200200564959

TI Evidence for a type III secretion system in *Aeromonas \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*.

AU Burr, Sarah E.; Stuber, Katja; Wahli, Thomas; \*\*\*Frey, Joachim\*\*\*  
 [Reprint author]

CS Institute of Veterinary Bacteriology, University of Berne,  
 Laenggassstrasse 122, CH-3012, Berne, Switzerland  
 joachim.frey@vbi.unibe.ch

SO Journal of Bacteriology, (November, 2002) Vol. 184, No. 21, pp. 5966-5970.  
 print.  
 CODEN: JOBAAY. ISSN: 0021-9193.

DT Article

LA English

ED Entered STN: 7 Nov 2002  
 Last Updated on STN: 7 Nov 2002

AB *Aeromonas \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*, the etiological agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against *yscV*, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of *Yersinia* species. Six of these proteins are encoded by genes comprising a *virA* operon. The *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* *yscV* homologue, *ascV*, was inactivated by marker replacement mutagenesis and used to generate an isogenic *ascV* mutant. Comparison of the extracellular protein profiles from the *ascV* mutant and the wild-type strain indicates that *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* secretes proteins via a type III secretion system. The recently identified ADP-ribosylating toxin *AexT* was identified as one such protein. Finally, we have compared the toxicities of the wild-type *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* strain and the *ascV* mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphological changes, including cell rounding, infection with the *ascV* mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.

L2 ANSWER 8 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 5

AN 2002:233045 BIOSIS

DN PREV200200233045

TI Characterization of an ADP-ribosyltransferase toxin (*AexT*) from *Aeromonas \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*.

AU Braun, Martin; Stuber, Katja; Schlatter, Yvonne; Wahli, Thomas; Kuhnert,

Peter; \*\*\*Frey, Joachim\*\*\* [Reprint author]  
 CS Institute for Veterinary Bacteriology, University of Bern, Langgassstrasse  
 122, CH-3012, Bern, Switzerland  
 joachim.frey@vbi.unibe.ch  
 SO Journal of Bacteriology, (April, 2002) Vol. 184, No. 7, pp. 1851-1858.  
 print.  
 CODEN: JOBAAY. ISSN: 0021-9193.  
 DT Article  
 LA English  
 ED Entered STN: 10 Apr 2002  
 Last Updated on STN: 10 Apr 2002  
 AB An ADP-ribosylating toxin named Aeromonas \*\*\*salmonicida\*\*\* exoenzyme  
 T (AexT) in A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*, the  
 etiological agent of furunculosis in fish, was characterized. Gene aexT,  
 encoding toxin AexT, was cloned and characterized by sequence analysis.  
 AexT shows significant sequence similarity to the ExoS and ExoT exotoxins  
 of Pseudomonas aeruginosa and to the YopE cytotoxin of different Yersinia  
 species. The aexT gene was detected in all of the 12 A.  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strains tested but was  
 absent from all other Aeromonas species. Recombinant AexT produced in  
 Escherichia coli possesses enzymatic ADP-ribosyltransferase activity.  
 Monospecific polyclonal antibodies directed against purified recombinant  
 AexT detected the toxin produced by A. \*\*\*salmonicida\*\*\* subsp.  
 \*\*\*salmonicida\*\*\* and cross-reacted with ExoS and ExoT of P. aeruginosa.  
 AexT toxin could be detected in a wild type (wt) strain of A.  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* freshly isolated from a  
 fish with furunculosis; however, its expression required contact with  
 RTG-2 rainbow trout gonad cells. Under these conditions, the AexT protein  
 was found to be intracellular or tightly cell associated. No AexT was  
 found when A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* was  
 incubated in cell culture medium in the absence of RTG-2 cells. Upon  
 infection with wt A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*,  
 the fish gonad RTG-2 cells rapidly underwent significant morphological  
 changes. These changes were demonstrated to constitute cell rounding,  
 which accompanied induction of production of AexT and which led to cell  
 lysis after extended incubation. An aexT mutant which was constructed  
 from the wt strain with an insertionally inactivated aexT gene by allelic  
 exchange had no toxic effect on RTG-2 cells and was devoid of AexT  
 production. Hence AexT is directly involved in the toxicity of A.  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* for RTG-2 fish cells.

=> e stuber katja/au

E1	66	STUBER K/AU
E2	1	STUBER KATHARINA/AU
E3	16	--> STUBER KATJA/AU
E4	3	STUBER KENT/AU
E5	2	STUBER KERSTIN/AU
E6	2	STUBER KLAUS/AU
E7	3	STUBER KURT/AU
E8	3	STUBER L/AU
E9	2	STUBER LARRY M/AU
E10	1	STUBER LOVELL S/AU
E11	308	STUBER M/AU
E12	2	STUBER M I/AU

=> s e1-e3 and salmonicida

L3 29 ("STUBER K"/AU OR "STUBER KATHARINA"/AU OR "STUBER KATJA"/AU)  
 AND SALMONICIDA

=> dup rem l3

PROCESSING COMPLETED FOR L3

L4 6 DUP REM L3 (23 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 6 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 6 USPATFULL on STN

AN 2005:68524 USPATFULL

TI , Novel type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\*, and

uses therefor

IN Frey, Joachim, Bern, SWITZERLAND  
 \*\*\*Stuber, Katja\*\*\*, Ittigen, SWITZERLAND  
 Thornton, Julian C., Victoria, CANADA  
 Kuzyk, Michael A., Richmond, CANADA  
 Burian, Jan, Victoria, CANADA

PA Universitat Bern (non-U.S. corporation)

PI US 2005058662 A1 20050317

AI US 2004-813908 A1 20040326 (10)

RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.  
 No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN

PRAI US 2000-248864P 20001115 (60)

DT Utility

FS APPLICATION

LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,  
 97204

CLMN Number of Claims: 15

ECL Exemplary Claim: CLM-01-22

DRWN 5 Drawing Page(s)

LN.CNT 1427

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a newly identified and characterized type III secretion  
 system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also  
 encompasses the use of components of the novel secretion system in  
 immunoprotection against *A. \*\*\*salmonicida\*\*\** infection, as well as  
 other diagnostic and therapeutic uses thereof.

L4 ANSWER 2 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 DUPLICATE 1

AN 2003:569435 BIOSIS

DN PREV200300562558

TI The ADP-ribosylating toxin, AexT, from *Aeromonas* \*\*\*salmonicida\*\*\*  
 subsp. \*\*\*salmonicida\*\*\* is translocated via a type III secretion  
 pathway.

AU Burr, Sarah E.; \*\*\*Stuber, Katja\*\*\*; Frey, Joachim [Reprint Author]

CS Institute of Veterinary Bacteriology, University of Berne,  
 Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland  
 joachim.frey@vbi.unibe.ch

SO Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591.  
 print.  
 CODEN: JOBAAY. ISSN: 0021-9193.

DT Article

LA English

ED Entered STN: 3 Dec 2003  
 Last Updated on STN: 3 Dec 2003

AB AexT is an extracellular ADP ribosyltransferase produced by the fish  
 pathogen *Aeromonas* \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*.  
 The protein is secreted by the bacterium via a recently identified type  
 III secretion system. In this study, we have identified a further 12 open  
 reading frames that possess high homology to genes encoding both  
 structural and regulatory components of the *Yersinia* type III secretion  
 apparatus. Using marker replacement mutagenesis of *aopB*, the *A.*  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* homologue of *yopB* in  
*Yersinia*, we demonstrate that the bacterium translocates the AexT toxin  
 directly into the cytosol of cultured fish cells via this type III  
 secretion pathway. An *acrV* mutant of *A. \*\*\*salmonicida\*\*\** subsp.  
 \*\*\*salmonicida\*\*\* displays a calcium-blind phenotype, expressing and  
 secreting significant amounts of AexT even in the presence of  $\text{CaCl}_2$   
 concentrations as high as 10 mM. This *acrV* mutant is also unable to  
 translocate AexT into the cytosol of fish cells, indicating *AcrV* is  
 involved in the translocation process. Inactivation of either the *aopB* or  
*acrV* gene in *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*  
 (resulting in an inability to translocate AexT) is accompanied by a loss  
 of cytotoxicity that can be restored by trans complementation. Finally,  
 we present data indicating that preincubation of the wild-type bacteria  
 with antibodies directed against recombinant *AcrV*-His protein provides  
 fish cells protection against the toxic effects of the bacterium.

L4 ANSWER 3 OF 6 CABA COPYRIGHT 2005 CABI on STN DUPLICATE 2

AN 2003:177812 CABA

DN 20033152569

TI Type III secretion genes in *Aeromonas* \*\*\*salmonicida\*\*\* subsp.  
 \*\*\*salmonicida\*\*\* are located on a large thermolabile virulence plasmid  
 AU \*\*\*Stuber, K.\*\*\* ; Burr, S. E.; Braun, M.; Wahli, T.; Frey, J.  
 CS Institute of Veterinary Bacteriology, University of Bern, Laenggassstrasse  
 122, Postfach, CH-3001 Bern, Switzerland. joachim.frey@vbi.unibe.ch  
 SO Journal of Clinical Microbiology, (2003) Vol. 41, No. 8, pp. 3854-3856. 6  
 ref.  
 Publisher: American Society for Microbiology (ASM). Washington  
 ISSN: 0095-1137  
 DOI: 10.1128/JCM.41.8.3854-3856.2003  
 CY United States  
 DT Journal  
 LA English  
 ED Entered STN: 20031107  
 Last Updated on STN: 20031107  
 AB Type III secretion genes in *Aeromonas* \*\*\*salmonicida\*\*\* subsp.  
 \*\*\*salmonicida\*\*\* are located on a large plasmid of approximately 140  
 kb. Cultivation of this organism at elevated temperatures such as 25[deg]C  
 can, however, result in loss of this plasmid. This is accompanied by a  
 loss of virulence for cultured fish cells.

L4 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN  
 AN 2002:391746 CAPLUS  
 DN 136:400588  
 TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in  
*Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines  
 IN Frey, Joachim; \*\*\*Stuber, Katja\*\*\* ; Thornton, Julian C.; Kuzyk,  
 Michael A.; Burian, Jan  
 PA Switz.  
 SO PCT Int. Appl., 39 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	CA 2467309	AA	20020523	CA 2001-2467309	20011115
	AU 2002014891	A5	20020527	AU 2002-14891	20011115
	GB 2385854	A1	20030903	GB 2003-13912	20011115
	NO 2003002217	A	20030709	NO 2003-2217	20030515
	US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion  
 system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses  
 the use of components of the novel secretion system in immunoprotection  
 against A. \*\*\*salmonicida\*\*\* infection, as well as other diagnostic  
 and therapeutic uses thereof. The components of type III secretion system  
 of *Aeromonas* \*\*\*salmonicida\*\*\* useful as fish vaccines are B cell  
 epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR,  
 AcrG, AcrV, and AcrH.

L4 ANSWER 5 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
 DUPLICATE 3  
 AN 2002:564959 BIOSIS  
 DN PREV200200564959  
 TI Evidence for a type III secretion system in *Aeromonas* \*\*\*salmonicida\*\*\*  
 subsp. \*\*\*salmonicida\*\*\*  
 AU Burr, Sarah E.; \*\*\*Stuber, Katja\*\*\* ; Wahli, Thomas; Frey, Joachim  
 [Reprint author]

CS Institute of Veterinary Bacteriology, University of Berne,  
Laenggassstrasse 122, CH-3012, Berne, Switzerland  
joachim.frey@vbi.unibe.ch

SO Journal of Bacteriology, (November, 2002) Vol. 184, No. 21, pp. 5966-5970.  
print.  
CODEN: JOBAAY. ISSN: 0021-9193.

DT Article  
LA English  
ED Entered STN: 7 Nov 2002  
Last Updated on STN: 7 Nov 2002

AB *Aeromonas* \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*, the  
etiological agent of furunculosis, is an important fish pathogen. We have  
screened this bacterium with a broad-host-range probe directed against  
yscV, the gene that encodes the archetype of a highly conserved family of  
inner membrane proteins found in every known type III secretion system.  
This has led to the identification of seven open reading frames that  
encode homologues to proteins functioning within the type III secretion  
systems of *Yersinia* species. Six of these proteins are encoded by genes  
comprising a virA operon. The *A. \*\*\*salmonicida\*\*\** subsp.  
\*\*\*salmonicida\*\*\* yscV homologue, ascV, was inactivated by marker  
replacement mutagenesis and used to generate an isogenic ascV mutant.  
Comparison of the extracellular protein profiles from the ascV mutant and  
the wild-type strain indicates that *A. \*\*\*salmonicida\*\*\** subsp.  
\*\*\*salmonicida\*\*\* secretes proteins via a type III secretion system. The  
recently identified ADP-ribosylating toxin AexT was identified as one such  
protein. Finally, we have compared the toxicities of the wild-type *A.*  
\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strain and the ascV  
mutant against RTG-2 rainbow trout gonad cells. While infection with the  
wild-type strain results in significant morphological changes, including  
cell rounding, infection with the ascV mutant has no toxic effect,  
indicating that the type III secretion system we have identified plays an  
important role in the virulence of this pathogen.

L4 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 4

AN 2002:233045 BIOSIS  
DN PREV200200233045

TI Characterization of an ADP-ribosyltransferase toxin (AexT) from *Aeromonas*  
\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*.

AU Braun, Martin; \*\*\*Stuber, Katja\*\*\*; Schlatter, Yvonne; Wahli, Thomas;  
Kuhnert, Peter; Frey, Joachim [Reprint author]

CS Institute for Veterinary Bacteriology, University of Bern, Langgassstrasse  
122, CH-3012, Bern, Switzerland  
joachim.frey@vbi.unibe.ch

SO Journal of Bacteriology, (April, 2002) Vol. 184, No. 7, pp. 1851-1858.  
print.  
CODEN: JOBAAY. ISSN: 0021-9193.

DT Article  
LA English  
ED Entered STN: 10 Apr 2002  
Last Updated on STN: 10 Apr 2002

AB An ADP-ribosylating toxin named *Aeromonas* \*\*\*salmonicida\*\*\* exoenzyme  
T (AexT) in *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*, the  
etiological agent of furunculosis in fish, was characterized. Gene aexT,  
encoding toxin AexT, was cloned and characterized by sequence analysis.  
AexT shows significant sequence similarity to the ExoS and ExoT exotoxins  
of *Pseudomonas aeruginosa* and to the YopE cytotoxin of different *Yersinia*  
species. The aexT gene was detected in all of the 12 *A.*  
\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strains tested but was  
absent from all other *Aeromonas* species. Recombinant AexT produced in  
*Escherichia coli* possesses enzymatic ADP-ribosyltransferase activity.  
Monospecific polyclonal antibodies directed against purified recombinant  
AexT detected the toxin produced by *A. \*\*\*salmonicida\*\*\** subsp.  
\*\*\*salmonicida\*\*\* and cross-reacted with ExoS and ExoT of *P. aeruginosa*.  
AexT toxin could be detected in a wild type (wt) strain of *A.*  
\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* freshly isolated from a  
fish with furunculosis; however, its expression required contact with  
RTG-2 rainbow trout gonad cells. Under these conditions, the AexT protein  
was found to be intracellular or tightly cell associated. No AexT was  
found when *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* was  
incubated in cell culture medium in the absence of RTG-2 cells. Upon



infection with wt A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* ,  
the fish gonad RTG-2 cells rapidly underwent significant morphological  
changes. These changes were demonstrated to constitute cell rounding,  
which accompanied induction of production of AexT and which led to cell  
lysis after extended incubation. An aexT mutant which was constructed  
from the wt strain with an insertionally inactivated aexT gene by allelic  
exchange had no toxic effect on RTG-2 cells and was devoid of AexT  
production. Hence AexT is directly involved in the toxicity of A.  
\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* for RTG-2 fish cells.

=> e thornton julian c/au

E1	8	THORNTON JUDITH M/AU
E2	3	THORNTON JULIAN/AU
E3	22 -->	THORNTON JULIAN C/AU
E4	1	THORNTON JULIAN CHARLES/AU
E5	17	THORNTON JULIE/AU
E6	1	THORNTON JULIE M/AU
E7	7	THORNTON JUSTIN/AU
E8	3	THORNTON JUSTIN A/AU
E9	166	THORNTON K/AU
E10	4	THORNTON K A/AU
E11	2	THORNTON K B/AU
E12	13	THORNTON K C/AU

=> s e2-e4 and salmonicida

L5 14 ("THORNTON JULIAN"/AU OR "THORNTON JULIAN C"/AU OR "THORNTON  
JULIAN CHARLES"/AU) AND SALMONICIDA

=> dup rem l5

PROCESSING COMPLETED FOR L5

L6 12 DUP REM L5 (2 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 12 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 12 USPATFULL on STN

AN 2005:68524 USPATFULL

TI Novel type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* , and  
uses therefor

IN Frey, Joachim, Bern, SWITZERLAND  
Stuber, Katja, Ittigen, SWITZERLAND  
\*\*\*Thornton, Julian C.\*\*\* , Victoria, CANADA  
Kuzyk, Michael A., Richmond, CANADA  
Burian, Jan, Victoria, CANADA

PA Universitat Bern (non-U.S. corporation)

PI US 2005058662 A1 20050317

AI US 2004-813908 A1 20040326 (10)

RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.  
No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN

PRAI US 2000-248864P 20001115 (60)

DT Utility

FS APPLICATION

LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,  
97204

CLMN Number of Claims: 15

ECL Exemplary Claim: CLM-01-22

DRWN 5 Drawing Page(s)

LN.CNT 1427

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a newly identified and characterized type III secretion  
system in Aeromonas \*\*\*salmonicida\*\*\* . The invention also  
encompasses the use of components of the novel secretion system in  
immunoprotection against A. \*\*\*salmonicida\*\*\* infection, as well as  
other diagnostic and therapeutic uses thereof.

L6 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391747 CAPLUS

DN 136:385049

TI Novel exoenzyme toxin of Aeromonas \*\*\*salmonicida\*\*\* and use for

recombinant salmon furunculosis vaccine  
 IN Frey, Joachim; Kuhnert, Peter; Braun, Martin; \*\*\*Thornton, Julian C.\*\*\*  
 ; Kuzik, Michael A.; Burian, Jan  
 PA Switz.  
 SO PCT Int. Appl., 38 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W:				
	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
	CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
	PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,				
	UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,				
	CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,				
	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 200202328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		
AB	A novel protein toxin named Aeromonas ***salmonicida*** exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of A. ***salmonicida***. Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against A. ***salmonicida*** infections.				

L6 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391746 CAPLUS

DN 136:400588

TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines

IN Frey, Joachim; Stuber, Katja; \*\*\*Thornton, Julian C.\*\*\* ; Kuzyk, Michael A.; Burian, Jan

PA Switz.

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W:				
	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
	CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
	PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,				
	UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,				
	CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,				
	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467309	AA	20020523	CA 2001-2467309	20011115
	AU 2002014891	A5	20020527	AU 2002-14891	20011115
	GB 2385854	A1	20030903	GB 2003-13912	20011115
	NO 2003002217	A	20030709	NO 2003-2217	20030515
	US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1589	W	20011115		
AB	Disclosed is a newly identified and characterized type III secretion system in Aeromonas ***salmonicida***. The invention also encompasses				

the use of components of the novel secretion system in immunoprotection against A. \*\*\*salmonicida\*\*\* infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of Aeromonas \*\*\*salmonicida\*\*\* useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

L6 ANSWER 4 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
AN 2001:333585 BIOSIS  
DN PREV200100333585

TI Physiological and immunological effects of adjuvanted Aeromonas  
\*\*\*salmonicida\*\*\* vaccines on juvenile rainbow trout.

AU Ackerman, Paige A. [Reprint author]; Iwama, George K.; \*\*\*Thornton,\*\*\*  
\*\*\* Julian C.\*\*\*

CS Faculty of Agricultural Sciences and Canadian Bacterial Diseases Network,  
University of British Columbia, Vancouver, BC, V6T 1Z4, Canada  
ackerman@interchange.ubc.ca

SO Journal of Aquatic Animal Health, (June, 2000) Vol. 12, No. 2, pp.  
157-164. print.  
ISSN: 0899-7659.

DT Article

LA English

ED Entered STN: 18 Jul 2001

Last Updated on STN: 19 Feb 2002

AB The effects of injectable vaccines against Aeromonas \*\*\*salmonicida\*\*\*  
on oxygen consumption, growth, kidney lysozyme activity, and anti-A.  
\*\*\*salmonicida\*\*\* plasma antibody titers of juvenile rainbow trout  
Oncorhynchus mykiss were examined. The vaccines were A.  
\*\*\*salmonicida\*\*\* bacterin only, bacterin adjuvanted with levamisole,  
bacterin in emulsified oil, microencapsulated bacterin, microencapsulated  
bacterin with muramyl dipeptide, microencapsulated bacterin with  
beta-1,3-glucan, and microencapsulated bacterin with Vibrio anguillarum  
lipopolysaccharide (LPS). The greatest and broadest ranges of responses  
were caused by the microencapsulated bacterin with V. anguillarum LPS.  
Oxygen consumption rates and specific growth rates were significantly  
higher over the course of 1 month among fish treated with the LPS vaccine.  
These fish also maintained a higher anti-A. \*\*\*salmonicida\*\*\* plasma  
antibody titer and kidney lysozyme activity for a substantially longer  
period than fish receiving the other treatments.

L6 ANSWER 5 OF 12 USPATFULL on STN

AN 96:20896 USPATFULL

TI Attenuated strains of Aeromonas \*\*\*salmonicida\*\*\* useful as fish  
vaccines

IN \*\*\*Thornton, Julian C.\*\*\*, Brentwood Bay, Canada  
Kay, William W., Victoria, Canada

PA University of Victoria, Victoria, Canada (non-U.S. corporation)

PI US 5498414 19960312

AI US 1992-957531 19921005 (7)

DT Utility

FS Granted

EXNAM Primary Examiner: Sidberry, Hazel F.; Assistant Examiner: Krsek-Staples,  
Julie

LREP Klarquist Sparkman Campbell Leigh & Whinston

CLMN Number of Claims: 34

ECL Exemplary Claim: 1

DRWN 7 Drawing Figure(s); 6 Drawing Page(s)

LN.CNT 1718

AB Novel attenuated strains of Aeromonas \*\*\*salmonicida\*\*\* are  
disclosed that are effective as live effective vaccines against  
furunculosis in fish. These vaccines may be administered by the  
immersion of fish in a solution of the vaccine. Methods of producing  
these strains and other strains having the identifying characteristics  
of these strains are also disclosed.

L6 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

AN 1996:409049 CAPLUS

DN 125:67377

TI The development of a live attenuated vaccine for the control of salmonid  
furunculosis (Aeromonas \*\*\*salmonicida\*\*\*, glucose catabolism,  
Oncorhynchus mykiss)

AU \*\*\*Thornton, Julian Charles\*\*\*  
CS Univ. of Victoria, Victoria, BC, Can.  
SO (1994) 165 pp. Avail.: Univ. Microfilms Int., Order No. DANN03527  
From: Diss. Abstr. Int., B 1996, 57(1), 105  
DT Dissertation  
LA English  
AB Unavailable

L6 ANSWER 7 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
AN 1994:86722 BIOSIS  
DN PREV199497099722  
TI Molecular biology of bacterial fish diseases.  
AU \*\*\*Thornton, Julian C.\*\*\* ; Garduno, Rafael A.; Kay, William W.  
CS Dep. Biochem. and Microbiol., Univ. Victoria, Victoria, BC V8W 3P6, Canada  
SO Hochachka, P. W. [Editor]; Mommsen, T. P. [Editor]. (1993) pp. 159-189.  
Biochemistry and Molecular Biology of Fishes; Molecular biology frontiers.  
Publisher: Elsevier Science Publishers B.V., PO Box 211, Sara  
Burgerhartstraat 25, 1000 AE Amsterdam, Netherlands; Elsevier Science  
Publishing Co., Inc., P.O. Box 882, Madison Square Station, New York, New  
York 10159-2101, USA. Series: Biochemistry and Molecular Biology of  
Fishes.  
ISBN: 0-444-81663-1.  
DT Book  
Book; (Book Chapter)  
General Review; (Literature Review)  
LA English  
ED Entered STN: 5 Mar 1994  
Last Updated on STN: 5 Mar 1994

L6 ANSWER 8 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 1  
AN 1993:586018 BIOSIS  
DN PREV199497005388  
TI Novel antigens expressed by Aeromonas \*\*\*salmonicida\*\*\* growth in  
vivo.  
AU \*\*\*Thornton, Julian C.\*\*\* ; Garduno, Rafael A.; Carlos, Steven J.; Kay,  
William W. [Reprint author]  
CS Dep. Biochem. Microbiol., Canadian Bacterial Diseases Network, Univ.  
Victoria, VIC, BC V8W 3P6, Canada  
SO Infection and Immunity, (1993) Vol. 61, No. 11, pp. 4582-4589.  
CODEN: INFIBR. ISSN: 0019-9567.  
DT Article  
LA English  
ED Entered STN: 28 Dec 1993  
Last Updated on STN: 28 Dec 1993  
AB Virulent and avirulent Aeromonas \*\*\*salmonicida\*\*\* strains grown  
inside intraperitoneal implants in Rainbow trout (*Oncorhynchus mykiss*)  
were examined for unique antigen expression. Western blots (immunoblots),  
performed with immune rabbit serum raised against in vivo-grown cells,  
revealed several unique antigens. With the exception of  
lipopolysaccharide (LPS), these novel antigens were destroyed after  
proteinase K treatment. The majority of these antigens were not induced  
in vitro in response to either iron limitation or anaerobiosis. In  
addition, electron microscopy demonstrated the presence of a putative  
capsule on in vivo-grown cells. Purification and fractionation of this  
carbohydrate material from cells grown in carbon-rich synthetic media  
resulted in the isolation and separation of an antigenically distinct LPS  
not seen with cells grown in standard media. Antiserum raised against in  
vivo-grown cells recognized both this LPS and the typical LPS of A.  
\*\*\*salmonicida\*\*\* apparent in in vitro-grown cells. Antiserum raised  
against in vitro-grown cells recognized only the LPS expressed in vitro.  
Antiserum directed against in vivo-grown cells was approximately 10 times  
more sensitive than serum directed against in vitro-grown cells in  
detecting A. \*\*\*salmonicida\*\*\* in infected fish kidney tissue.

L6 ANSWER 9 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 2  
AN 1993:479464 BIOSIS  
DN PREV199396113064  
TI Aeromonas \*\*\*salmonicida\*\*\* grown in vivo.  
AU Garduno, Rafael A.; \*\*\*Thornton, Julian C.\*\*\* ; Kay, William W.

[Reprint author]

CS Canadian Bacterial Disease Network, Univ. Victoria, P.O. Box 3055,  
Victoria, British Columbia V8W 3P6, Canada

SO Infection and Immunity, (1993) Vol. 61, No. 9, pp. 3854-3862.  
CODEN: INFIBR. ISSN: 0019-9567.

DT Article

LA English

ED Entered STN: 22 Oct 1993  
Last Updated on STN: 22 Oct 1993

AB The virulent fish pathogen *Aeromonas* \*\*\*salmonicida\*\*\* was rapidly  
killed in vivo when restricted inside a diffusion chamber implanted  
intraperitoneally in rainbow trout. After a period of regrowth, the  
survivors had acquired resistance to host-mediated bacteriolysis,  
phagocytosis, and oxidative killing, properties which were subsequently  
lost by growth in vitro. Resistance to bacteriolysis and phagocytosis was  
associated with a newly acquired capsular layer revealed by acidic  
polysaccharide staining and electron microscopy. This capsular layer  
shielded the underlying, regular surface array (S-layer) from immunogold  
labeling with a primary antibody to the S-layer protein. Resistance to  
oxidative killing was mediated by a mechanism not associated with the  
presence of the capsular layer. An attenuated vaccine strain of *A.*  
\*\*\*salmonicida\*\*\* grown in vivo failed to express the capsular layer.  
Consequently, the in vivo-grown cells of this attenuated strain remained  
as sensitive to bacteriolysis, and as avidly adherent to macrophages, as  
the in vitro-grown cells. The importance of these new virulence  
determinants and their relation to the known virulence factors of *A.*  
\*\*\*salmonicida\*\*\* are discussed.

L6 ANSWER 10 OF 12 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on  
STN

AN 1994:77914 BIOSIS

DN PREV199497090914

TI Fate of the fish pathogen *Aeromonas* \*\*\*salmonicida\*\*\* in the  
peritoneal cavity of rainbow trout.

AU Garduno, Rafael A.; \*\*\*Thornton, Julian C.\*\*\* ; Kay, William W.  
[Reprint author]

CS Dep. Biochem. and Microbiol., Univ. Victoria, Petch Bldg., P.O. Box 3055,  
Victoria, BC V8W 3P6, Canada

SO Canadian Journal of Microbiology, (1993) Vol. 39, No. 11, pp. 1051-1058.  
CODEN: CJMIAZ. ISSN: 0008-4166.

DT Article

LA English

ED Entered STN: 22 Feb 1994  
Last Updated on STN: 22 Feb 1994

AB A model was developed to study the fate of the fish pathogen *Aeromonas*  
\*\*\*salmonicida\*\*\* in vivo, inside a specialized intraperitoneal chamber  
implanted in rainbow trout, *Oncorhynchus mykiss*. Although normally  
recalcitrant to lytic agents in vitro, owing to the presence of its  
regular surface array (S layer), *A.* \*\*\*salmonicida\*\*\* was rapidly  
killed in the peritoneal cavity by a host-derived, soluble lytic activity  
present in peritoneal fluid. Peritoneal fluid was also found to kill  
other bacteria and lyse various types of erythrocytes, but was  
particularly lytic to *A.* \*\*\*salmonicida\*\*\*. Intraperitoneal survival  
of injected (free) *A.* \*\*\*salmonicida\*\*\* cells was several orders of  
magnitude higher than survival of implanted (restrained) cells. Injected  
free cells could evade the lytic activity of peritoneal fluid because they  
readily spread, initiating lethal infections. One evasion strategy was  
envisioned to be the penetration of peritoneal and (or) tissue  
macrophages. In spite of the killing mechanisms of these phagocytic  
cells, *A.* \*\*\*salmonicida\*\*\* was still able to survive and even  
replicate inside head kidney macrophages, thereby supporting the notion of  
*A.* \*\*\*salmonicida\*\*\* as a facultatively intracellular pathogen.  
Intraperitoneal chambers in rainbow trout may constitute a valuable  
experimental tool for studying the in vivo fate of *A.* \*\*\*salmonicida\*\*\*  
, and perhaps of other fish pathogens as well.

L6 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

AN 1994:553182 CAPLUS

DN 121:153182

TI Does the S-layer of *Aeromonas* \*\*\*salmonicida\*\*\* exist in more than one  
functional organizational state?

AU Garduno, Rafael A.; \*\*\*Thornton, Julian C.\*\*\* ; Kay, William W.  
 CS Department Biochemistry and Microbiology and Canadian Bacterial Disease  
 Network, University Victoria, Victoria, BC, Can.  
 SO NATO ASI Series, Series A: Life Sciences (1993), 252(ADVANCES IN  
 BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 285-8  
 CODEN: NALSDJ; ISSN: 0258-1213  
 DT Journal  
 LA English  
 AB The S-layer of Aeromonas \*\*\*salmonicida\*\*\* possesses significant  
 flexibility and plasticity. The functional relevance of one of these  
 novel structural patterns (big squares pattern) was studied here. The big  
 squares pattern was effective in protecting cells from complement-mediated  
 bacteriolysis and oxidative killing; this pattern also mediated a very  
 efficient assocn. of Aeromonas with macrophage and the hemin anal Congo  
 Red. Host-mediated structural changes in the S-layer may be involved in  
 pathogenesis.

L6 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2005 ACS on STN

AN 1994:529448 CAPLUS

DN 121:129448

TI Structure - function aspects of the Aeromonas \*\*\*salmonicida\*\*\*  
 S-layer

AU Kay, William W.; \*\*\*Thornton, Julian C.\*\*\* ; Garduno, Raphael A.  
 CS Department Biochemistry and Microbiology, University Victoria, Victoria,  
 BC, Can.

SO NATO ASI Series, Series A: Life Sciences (1993), 252(ADVANCES IN  
 BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 151-8  
 CODEN: NALSDJ; ISSN: 0258-1213

DT Journal; General Review

LA English

AB A review with 22 refs.

=> e kuzyk michael a/au

E1 1 KUZYK MARK GEORGE/AU  
 E2 3 KUZYK MICHAEL/AU  
 E3 16 --> KUZYK MICHAEL A/AU  
 E4 1 KUZYK MICHAEL ALLAN/AU  
 E5 1 KUZYK O V/AU  
 E6 8 KUZYK P/AU  
 E7 1 KUZYK P H/AU  
 E8 1 KUZYK PAUL R/AU  
 E9 2 KUZYK PAVLO/AU  
 E10 2 KUZYK PETER/AU  
 E11 2 KUZYK R/AU  
 E12 3 KUZYK R W/AU

=> s e2-e4 and salmonicida

L7 4 ("KUZYK MICHAEL"/AU OR "KUZYK MICHAEL A"/AU OR "KUZYK MICHAEL  
 ALLAN"/AU) AND SALMONICIDA

=> dup rem l7

PROCESSING COMPLETED FOR L7

L8 3 DUP REM L7 (1 DUPLICATE REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L8 ANSWER 1 OF 3 USPATFULL on STN

AN 2005:68524 USPATFULL

TI Novel type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* , and  
 uses therefor

IN Frey, Joachim, Bern, SWITZERLAND  
 Stuber, Katja, Ittigen, SWITZERLAND  
 Thornton, Julian C., Victoria, CANADA  
 \*\*\*Kuzyk, Michael A.\*\*\* , Richmond, CANADA  
 Burian, Jan, Victoria, CANADA

PA Universitat Bern (non-U.S. corporation)

PI US 2005058662 A1 20050317

AI US 2004-813908 A1 20040326 (10)

RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.  
No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN  
PRAI US 2000-248864P 20001115 (60)  
DT Utility  
FS APPLICATION  
LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,  
97204  
CLMN Number of Claims: 15  
ECL Exemplary Claim: CLM-01-22  
DRWN 5 Drawing Page(s)  
LN.CNT 1427

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a newly identified and characterized type III secretion  
system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also  
encompasses the use of components of the novel secretion system in  
immunoprotection against *A. \*\*\*salmonicida\*\*\** infection, as well as  
other diagnostic and therapeutic uses thereof.

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391746 CAPLUS

DN 136:400588

TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in  
*Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines

IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; \*\*\*Kuzyk, Michael\*\*\*  
\*\*\* A.\*\*\* ; Burian, Jan

PA Switz.

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W:				
	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,				
	CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,				
	GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,				
	LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,				
	PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,				
	UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,				
	CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,				
	BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	CA 2467309	AA	20020523	CA 2001-2467309	20011115
	AU 2002014891	A5	20020527	AU 2002-14891	20011115
	GB 2385854	A1	20030903	GB 2003-13912	20011115
	NO 2003002217	A	20030709	NO 2003-2217	20030515
	US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion  
system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses  
the use of components of the novel secretion system in immunoprotection  
against *A. \*\*\*salmonicida\*\*\** infection, as well as other diagnostic  
and therapeutic uses thereof. The components of type III secretion system  
of *Aeromonas* \*\*\*salmonicida\*\*\* useful as fish vaccines are B cell  
epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR,  
AcrG, AcrV, and AcrH.

L8 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 1

AN 1998:98617 BIOSIS

DN PREV199800098617

TI Structural and physiological determinants of resistance of *Aeromonas*  
\*\*\*salmonicida\*\*\* to reactive radicals.

AU Garduno, Rafael A.; \*\*\*Kuzyk, Michael A.\*\*\* ; Kay, William W. [Reprint  
author]

CS Dep. Biochemistry Microbiol., Canadian Bacterial Disease Network, Univ.  
Victoria, P.O. Box 3055, Victoria, BC V8W 3P6, Canada

SO Canadian Journal of Microbiology, (Nov., 1997) Vol. 43, No. 11, pp.

1044-1053. print.  
CODEN: CJMIAZ. ISSN: 0008-4166.

DT Article  
LA English  
ED Entered STN: 25 Feb 1998  
Last Updated on STN: 25 Feb 1998

AB The facultative intracellular pathogen *Aeromonas* \*\*\*salmonicida\*\*\* survives and replicates in macrophages, a virulence trait presumed to be associated with its ability to resist reactive radicals. The mechanisms used by A. \*\*\*salmonicida\*\*\* to resist reactive radicals in vitro were shown to have both structural and physiological determinants. The sensitivity of A. \*\*\*salmonicida\*\*\* to exogenous H<sub>2</sub>O<sub>2</sub>, superoxide, and nitrogen radicals, as well as endogenous oxygen radicals, differed depending on growth conditions, cell surface structure, and preexposure to sublethal doses of radicals. Whereas sensitivities to exogenous oxygen radicals did not correlate with basal levels of catalase or Fe-superoxide dismutase, under similar culture conditions S-layer positive cells were more resistant to oxygen radicals than S-layer mutants. S-layer mutants recovered resistance when physically reconstituted with S-layer sheets. Hemin-coated S-layers, while protective against nitrogen radicals, sensitized A. \*\*\*salmonicida\*\*\* to H<sub>2</sub>O<sub>2</sub>. Sublethal concentrations of H<sub>2</sub>O<sub>2</sub> or superoxide induced a highly protective response characterized by de novo synthesis of both catalase and Mn-superoxide dismutase. It is proposed that for A. \*\*\*salmonicida\*\*\* the constitutive S-layer provides a first line of defense and the inducible catalase and Mn-superoxide dismutase provide a powerful second line of defense against macrophage-mediated killing via reactive oxygen species.

=> e burian jan/au

E1	3	BURIAN J A/AU
E2	1	BURIAN JAMES E/AU
E3	42 -->	BURIAN JAN/AU
E4	1	BURIAN JAROSLAW/AU
E5	1	BURIAN JEEMUSU TOMUPUSON/AU
E6	2	BURIAN JIEEMUSU EDOWAAZU/AU
E7	1	BURIAN JIEEMUSU TETSUDOMAASHIYU/AU
E8	1	BURIAN JIEEMUSU TOOMASU/AU
E9	1	BURIAN JIEI BIIRIYUU/AU
E10	1	BURIAN JIEI SURIIBAN/AU
E11	11	BURIAN JIRI/AU
E12	1	BURIAN JIYOJIFU ROBO/AU

=> s e3 and salmonicida

L9 3 "BURIAN JAN"/AU AND SALMONICIDA

=> dup rem l9

PROCESSING COMPLETED FOR L9

L10 3 DUP REM L9 (0 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L10 ANSWER 1 OF 3 USPATFULL on STN

AN 2005:68524 USPATFULL

TI Novel type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\*, and uses therefor

IN Frey, Joachim, Bern, SWITZERLAND  
Stuber, Katja, Ittigen, SWITZERLAND  
Thornton, Julian C., Victoria, CANADA  
Kuzyk, Michael A., Richmond, CANADA  
\*\*\*Burian, Jan\*\*\*, Victoria, CANADA

PA Universitat Bern (non-U.S. corporation)

PI US 2005058662 A1 20050317

AI US 2004-813908 A1 20040326 (10)

RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.  
No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN

PRAI US 2000-248864P 20001115 (60)

DT Utility

FS APPLICATION

LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,



97204

CLMN Number of Claims: 15  
ECL Exemplary Claim: CLM-01-22  
DRWN 5 Drawing Page(s)  
LN.CNT 1427

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a newly identified and characterized type III secretion system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses the use of components of the novel secretion system in immunoprotection against *A.\*\*\*salmonicida\*\*\** infection, as well as other diagnostic and therapeutic uses thereof.

L10 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391747 CAPLUS

DN 136:385049

TI Novel exoenzyme toxin of *Aeromonas* \*\*\*salmonicida\*\*\* and use for recombinant salmon furunculosis vaccine

IN Frey, Joachim; Kuhnert, Peter; Braun, Martin; Thornton, Julian C.; Kuzik, Michael A.; \*\*\*Burian, Jan\*\*\*

PA Switz.

SO PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040515	A2	20020523	WO 2001-CA1600	20011115
	WO 2002040515	A3	20021227		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
	CA 2467360	AA	20020523	CA 2001-2467360	20011115
	AU 2002023328	A5	20020527	AU 2002-23328	20011115
	GB 2389112	A1	20031203	GB 2003-13840	20011115
	NO 2003002218	A	20030714	NO 2003-2218	20030515
PRAI	US 2000-248864P	P	20001115		
	WO 2001-CA1600	W	20011115		

AB A novel protein toxin named *Aeromonas* \*\*\*salmonicida\*\*\* exoenzyme T (AexT), which belongs to the family of ADP-ribosylating toxins, is disclosed as is a novel Calcium (or other cation concn.) dependent promoter of *A.\*\*\*salmonicida\*\*\**. Also disclosed are diagnostic, preventative, and therapeutic techniques, including the prepn. of traditional, recombinant, and improved bacterin vaccines based on AexT for inducing immunity against *A.\*\*\*salmonicida\*\*\** infections.

L10 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:391746 CAPLUS

DN 136:400588

TI T or B cell epitopes of AcrV or AcrD of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines

IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; \*\*\*Burian, Jan\*\*\*

PA Switz.

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
	WO 2002040514	A3	20021227		
	W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,			

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,  
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,  
UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,  
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,  
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses the use of components of the novel secretion system in immunoprotection against *A. \*\*\*salmonicida\*\*\** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of *Aeromonas \*\*\*salmonicida\*\*\** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. Acr1, Acr2, Acr3, Acr4, AcrD, AcrR, AcrG, AcrV, and AcrH.

=> s salmonicida  
L11 7273 SALMONICIDA

=> s l11 and acr?  
L12 258 L11 AND ACR?

=>

=> dup rem l12  
PROCESSING COMPLETED FOR L12  
L13 193 DUP REM L12 (65 DUPLICATES REMOVED)

=> s l13 and ((acr1)or(acr2)or(acr3)or(acr4)or(acrd)or(acrr)or(acrg)or(acrv)or(acrh))  
L14 27 L13 AND ((ACR1) OR(ACR2) OR(ACR3) OR(ACR4) OR(ACRD) OR(ACRR) OR(ACRG) OR(ACRV) OR(ACRH))

=> d bib ab kwic 1-  
YOU HAVE REQUESTED DATA FROM 27 ANSWERS - CONTINUE? Y/(N):y

L14 ANSWER 1 OF 27 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
AN 2003:569435 BIOSIS  
DN PREV200300562558  
TI The ADP-ribosylating toxin, AexT, from *Aeromonas \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* is translocated via a type III secretion pathway.  
AU Burr, Sarah E.; Stuber, Katja; Frey, Joachim [Reprint Author]  
CS Institute of Veterinary Bacteriology, University of Berne, Laenggassstrasse 122, CH-3001, Postfach, Berne, Switzerland  
joachim.frey@vbi.unibe.ch  
SO Journal of Bacteriology, (November 2003) Vol. 185, No. 22, pp. 6583-6591.  
print.  
CODEN: JOBAAAY. ISSN: 0021-9193.  
DT Article  
LA English  
ED Entered STN: 3 Dec 2003  
Last Updated on STN: 3 Dec 2003

AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen *Aeromonas \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\*. The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have identified a further 12 open reading frames that possess high homology to genes encoding both structural and regulatory components of the *Yersinia* type III secretion apparatus. Using marker replacement mutagenesis of *aopB*, the *A. \*\*\*salmonicida\*\*\** subsp. \*\*\*salmonicida\*\*\* homologue of *yopB* in *Yersinia*, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An \*\*\*acrV\*\*\* mutant of *A. \*\*\*salmonicida\*\*\**

subsp. **\*\*\*salmonicida\*\*\*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl<sub>2</sub> concentrations as high as 10 mM. This **\*\*\*acrV\*\*\*** mutant is also unable to translocate AexT into the cytosol of fish cells, indicating **\*\*\*AcrV\*\*\*** is involved in the translocation process. Inactivation of either the *aopB* or **\*\*\*acrV\*\*\*** gene in *A. \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant **\*\*\*AcrV\*\*\***-His protein provides fish cells protection against the toxic effects of the bacterium.

TI The ADP-ribosylating toxin, AexT, from *Aeromonas \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\*** is translocated via a type III secretion pathway.

AB AexT is an extracellular ADP ribosyltransferase produced by the fish pathogen *Aeromonas \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\***. The protein is secreted by the bacterium via a recently identified type III secretion system. In this study, we have. . . both structural and regulatory components of the *Yersinia* type III secretion apparatus. Using marker replacement mutagenesis of *aopB*, the *A. \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\*** homologue of *yopB* in *Yersinia*, we demonstrate that the bacterium translocates the AexT toxin directly into the cytosol of cultured fish cells via this type III secretion pathway. An **\*\*\*acrV\*\*\*** mutant of *A. \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\*** displays a calcium-blind phenotype, expressing and secreting significant amounts of AexT even in the presence of CaCl<sub>2</sub> concentrations as high as 10 mM. This **\*\*\*acrV\*\*\*** mutant is also unable to translocate AexT into the cytosol of fish cells, indicating **\*\*\*AcrV\*\*\*** is involved in the translocation process. Inactivation of either the *aopB* or **\*\*\*acrV\*\*\*** gene in *A. \*\*\*salmonicida\*\*\** subsp. **\*\*\*salmonicida\*\*\*** (resulting in an inability to translocate AexT) is accompanied by a loss of cytotoxicity that can be restored by trans complementation. Finally, we present data indicating that preincubation of the wild-type bacteria with antibodies directed against recombinant **\*\*\*AcrV\*\*\***-His protein provides fish cells protection against the toxic effects of the bacterium.

IT . . . Concepts  
Infection; Molecular Genetics (Biochemistry and Molecular Biophysics); Toxicology

IT Parts, Structures, & Systems of Organisms  
cytosol

IT Diseases  
*Aeromonas \*\*\*salmonicida\*\*\** **\*\*\*salmonicida\*\*\*** infection: bacterial disease

IT Chemicals & Biochemicals  
AexT: ADP ribosyltransferase, toxin; calcium chloride; open reading frame; recombinant **\*\*\*AcrV\*\*\***-His protein

ORGN Classifier  
*Aeromonadaceae* 06701  
Super Taxa  
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms  
Organism Name  
*Aeromonas \*\*\*salmonicida\*\*\** **\*\*\*salmonicida\*\*\*** (subspecies): pathogen  
Taxa Notes  
Bacteria, Eubacteria, Microorganisms

ORGN Classifier  
*Pisces* 85200  
Super Taxa  
Vertebrata; Chordata; Animalia  
Organism Name  
fish (common):. . .

GEN *Aeromonas \*\*\*salmonicida\*\*\** **\*\*\*salmonicida\*\*\*** **\*\*\*acrV\*\*\*** gene  
(*Aeromonadaceae*); *Aeromonas \*\*\*salmonicida\*\*\** **\*\*\*salmonicida\*\*\***  
*aopB* gene (*Aeromonadaceae*)

L14 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2005 ACS on STN

AN 2002:806113 CAPLUS

DN 138:86215

TI Evidence for a type III secretion system in *Aeromonas \*\*\*salmonicida\*\*\**

subsp. \*\*\*salmonicida\*\*\*

AU Burr, Sarah E.; Stuber, Katja; Wahli, Thomas; Frey, Joachim

CS Institute of Veterinary Bacteriology and Centre for Fish and Wildlife Health, University of Berne, Bern, CH-3012, Switz.

SO Journal of Bacteriology (2002), 184(21), 5966-5970  
CODEN: JOBAAAY; ISSN: 0021-9193

PB American Society for Microbiology

DT Journal

LA English

AB Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*, the etiol. agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* yscV homolog, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* secretes proteins via a type III secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphol. changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.

RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Evidence for a type III secretion system in Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*

AB Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\*, the etiol. agent of furunculosis, is an important fish pathogen. We have screened this bacterium with a broad-host-range probe directed against yscV, the gene that encodes the archetype of a highly conserved family of inner membrane proteins found in every known type III secretion system. This has led to the identification of seven open reading frames that encode homologues to proteins functioning within the type III secretion systems of Yersinia species. Six of these proteins are encoded by genes comprising a virA operon. The A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* yscV homolog, ascV, was inactivated by marker replacement mutagenesis and used to generate an isogenic ascV mutant. Comparison of the extracellular protein profiles from the ascV mutant and the wild-type strain indicates that A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* secretes proteins via a type III secretion system. The recently identified ADP-ribosylating toxin AexT was identified as one such protein. Finally, we have compared the toxicities of the wild-type A. \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* strain and the ascV mutant against RTG-2 rainbow trout gonad cells. While infection with the wild-type strain results in significant morphol. changes, including cell rounding, infection with the ascV mutant has no toxic effect, indicating that the type III secretion system we have identified plays an important role in the virulence of this pathogen.

IT Gene, microbial  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
( \*\*\*acr1\*\*\* ; evidence for a type III secretion system in Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )

IT Gene, microbial  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
( \*\*\*acr2\*\*\* ; evidence for a type III secretion system in Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )

IT Gene, microbial  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
( \*\*\*acrG\*\*\* ; evidence for a type III secretion system in Aeromonas

\*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 ( \*\*\*acrR\*\*\* ; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (aopN; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (ascN; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (ascV; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Gene, microbial  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (ascX; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Aeromonas \*\*\*salmonicida\*\*\* \*\*\*salmonicida\*\*\*  
 DNA sequences  
 Protein sequences  
 Secretion (process)  
 Virulence (microbial)  
 (evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT ADP ribosylation factor  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene \*\*\*acr1\*\*\* ; evidence for a type III secretion system in  
 Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene \*\*\*acr2\*\*\* ; evidence for a type III secretion system in  
 Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene \*\*\*acrG\*\*\* ; evidence for a type III secretion system in  
 Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene \*\*\*acrR\*\*\* ; evidence for a type III secretion system in  
 Aeromonas \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene aopN; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)  
 (gene ascN; evidence for a type III secretion system in Aeromonas  
 \*\*\*salmonicida\*\*\* subsp. \*\*\*salmonicida\*\*\* )  
 IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
 (Biological study)

(gene ascV; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
 (gene ascX; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

IT Proteins  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
 (gene ascY; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

IT Operon  
 (virA; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

IT 484256-36-2 484256-37-3 484256-38-4 484256-39-5 484256-40-8  
 484256-41-9 484256-42-0 484256-43-1 484256-44-2  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
 (amino acid sequence; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

IT 465948-29-2  
 RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
 (nucleotide sequence; evidence for a type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** subsp. **\*\*\*salmonicida\*\*\*** )

L14 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2005 ACS on STN  
 AN 2002:391746 CAPLUS  
 DN 136:400588  
 TI T or B cell epitopes of **\*\*\*AcrV\*\*\*** or **\*\*\*AcrD\*\*\*** of type III secretion pathway in *Aeromonas* **\*\*\*salmonicida\*\*\*** for use as vaccines  
 IN Frey, Joachim; Stuber, Katja; Thornton, Julian C.; Kuzyk, Michael A.; Burian, Jan  
 PA Switz.  
 SO PCT Int. Appl., 39 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
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WO 2002040514	A2	20020523	WO 2001-CA1589	20011115
WO 2002040514	A3	20021227		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
CA 2467309	AA	20020523	CA 2001-2467309	20011115
AU 2002014891	A5	20020527	AU 2002-14891	20011115
GB 2385854	A1	20030903	GB 2003-13912	20011115
NO 2003002217	A	20030709	NO 2003-2217	20030515
US 2005058662	A1	20050317	US 2004-813908	20040326
PRAI US 2000-248864P	P	20001115		
WO 2001-CA1589	W	20011115		

AB Disclosed is a newly identified and characterized type III secretion system in *Aeromonas* **\*\*\*salmonicida\*\*\*** . The invention also encompasses the use of components of the novel secretion system in immunoprotection against *A. \*\*\*salmonicida\*\*\** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of *Aeromonas* **\*\*\*salmonicida\*\*\*** useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. **\*\*\*Acr1\*\*\*** , **\*\*\*Acr2\*\*\*** , **\*\*\*Acr3\*\*\*** , **\*\*\*Acr4\*\*\*** , **\*\*\*AcrD\*\*\*** , **\*\*\*AcrR\*\*\*** , **\*\*\*AcrG\*\*\*** , **\*\*\*AcrV\*\*\*** , and **\*\*\*AcrH\*\*\*** .

TI T or B cell epitopes of **\*\*\*AcrV\*\*\*** or **\*\*\*AcrD\*\*\*** of type III secretion pathway in *Aeromonas* **\*\*\*salmonicida\*\*\*** for use as vaccines

AB Disclosed is a newly identified and characterized type III secretion system in *Aeromonas* \*\*\*salmonicida\*\*\*. The invention also encompasses the use of components of the novel secretion system in immunoprotection against *A.\*\*\*salmonicida\*\*\** infection, as well as other diagnostic and therapeutic uses thereof. The components of type III secretion system of *Aeromonas* \*\*\*salmonicida\*\*\* useful as fish vaccines are B cell epitopes or T cell epitopes, e.g. \*\*\*Acr1\*\*\*, \*\*\*Acr2\*\*\*, \*\*\*Acr3\*\*\*, \*\*\*Acr4\*\*\*, \*\*\*AcrD\*\*\*, \*\*\*AcrR\*\*\*, \*\*\*AcrG\*\*\*, \*\*\*AcrV\*\*\*, and \*\*\*AcrH\*\*\*.

ST *Aeromonas* \*\*\*salmonicida\*\*\* \*\*\*AcrD\*\*\* \*\*\*AcrV\*\*\* epitope vaccine

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*Acr1\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*Acr2\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*Acr3\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*Acr4\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*AcrG\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*AcrH\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*AcrR\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) ( \*\*\*AcrV\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
 RL: BSU (Biological study, unclassified); DGN (Diagnostic use); THU (Therapeutic use); BIOL (Biological study); USES (Uses) (AexT; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT *Aeromonas* \*\*\*salmonicida\*\*\*  
 Antiserums  
 Blood serum  
 Culture media  
 Drug delivery systems

Epitopes  
Fish  
Genetic vectors  
Oncorhynchus mykiss  
Plasmids  
Spraying  
Susceptibility (genetic)  
Vaccines

(T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Antibodies and Immunoglobulins

RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)

(T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Gene, microbial

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Oligonucleotides

Probes (nucleic acid)

RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Gene, microbial

RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP

(Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

( \*\*\*acrD\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Gene, microbial

RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP

(Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

( \*\*\*acrV\*\*\* ; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Immunostimulants

(adjuvants; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Gene, microbial

RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP

(Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(aexT; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Diagnosis

(agents; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT B cell (lymphocyte)

T cell (lymphocyte)

(epitope; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Animal tissue

(fish; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Microorganism

(host cell; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems

(immersion; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of



type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems  
(injections, i.m.; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems  
(injections, i.p.; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Matrix media  
(insol.; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems  
(intracellular; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems  
(intradermal; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Drug delivery systems  
(oral; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Mutagenesis  
(site-directed, addn.; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Mutagenesis  
(site-directed, deletion; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Mutagenesis  
(site-directed, substitution; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT ADP ribosylation factor  
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(toxin; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT Proteins  
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(type III secretion app.; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT 58319-92-9, Exoenzyme T  
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(*Aeromonas* \*\*\*salmonicida\*\*\*; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT 429112-86-7 429112-88-9 429113-04-2 429113-21-3 429113-22-4 429113-31-5 429113-34-8 429113-35-9 429113-36-0  
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(amino acid sequence; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT 429113-37-1  
RL: BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(nucleotide sequence; T or B cell epitopes of \*\*\*AcrV\*\*\* or \*\*\*AcrD\*\*\* of type III secretion pathway in *Aeromonas* \*\*\*salmonicida\*\*\* for use as vaccines)

IT 429115-35-5 429115-36-6 429115-37-7 429115-38-8 429115-39-9 429115-40-2 429115-41-3 429115-42-4 429115-43-5

RL: PRP (Properties)  
(unclaimed sequence; t or B cell epitopes of \*\*\*AcrV\*\*\* or  
\*\*\*AcrD\*\*\* of type III secretion pathway in Aeromonas  
\*\*\*salmonicida\*\*\* for use as vaccines)

L14 ANSWER 4 OF 27 USPATFULL on STN  
AN 2005:68524 USPATFULL  
TI Novel type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* , and  
uses therefor  
IN Frey, Joachim, Bern, SWITZERLAND  
Stuber, Katja, Ittigen, SWITZERLAND  
Thornton, Julian C., Victoria, CANADA  
Kuzyk, Michael A., Richmond, CANADA  
Burian, Jan, Victoria, CANADA  
PA Universitat Bern (non-U.S. corporation)  
PI US 2005058662 A1 20050317  
AI US 2004-813908 A1 20040326 (10)  
RLI Continuation of Ser. No. US 416902, PENDING A 371 of International Ser.  
No. WO 2001-CA1589, filed on 15 Nov 2001, UNKNOWN  
PRAI US 2000-248864P 20001115 (60)  
DT Utility  
FS APPLICATION  
LREP KLARQUIST SPARKMAN, LLP, 121 SW SALMON STREET, SUITE 1600, PORTLAND, OR,  
97204  
CLMN Number of Claims: 15  
ECL Exemplary Claim: CLM-01-22  
DRWN 5 Drawing Page(s)  
LN.CNT 1427  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB Disclosed is a newly identified and characterized type III secretion  
system in Aeromonas \*\*\*salmonicida\*\*\* . The invention also  
encompasses the use of components of the novel secretion system in  
immunoprotection against A. \*\*\*salmonicida\*\*\* infection, as well as  
other diagnostic and therapeutic uses thereof.  
TI Novel type III secretion pathway in Aeromonas \*\*\*salmonicida\*\*\* , and  
uses therefor  
AB Disclosed is a newly identified and characterized type III secretion  
system in Aeromonas \*\*\*salmonicida\*\*\* . The invention also  
encompasses the use of components of the novel secretion system in  
immunoprotection against A. \*\*\*salmonicida\*\*\* infection, as well as  
other diagnostic and therapeutic uses thereof.  
SUMM . . . relates to bacterial secretion systems, and in particular to a  
newly identified and characterized type III secretion system in  
Aeromonas \*\*\*salmonicida\*\*\* . The invention also encompasses the use  
of components of the novel secretion system in immunoprotection against  
A. \*\*\*salmonicida\*\*\* infection, as well as other diagnostic and  
therapeutic uses thereof.  
SUMM [0003] Aeromonas \*\*\*salmonicida\*\*\* , a Gram-negative, facultatively  
anaerobic, non-motile, rod shaped bacterium, growing at temperatures  
around 20.degree. C., is the etiological agent of furunculoses. . .  
SUMM [0005] Knowledge of the mechanisms of pathogenicity of A.  
\*\*\*salmonicida\*\*\* , and in particular of the main virulence factors  
involved, is essential in the development of efficient strategies to  
prevent outbreaks of furunculoses caused by A. \*\*\*salmonicida\*\*\* .  
Currently, several potential virulence factors of A. \*\*\*salmonicida\*\*\*  
have been reported, including a surface-layer protein (Chu et al.,  
1991), the hemolysins ASH1, ASH3, ASH4 (Hirono and Aoki, 1993), . . .  
is unclear and many of them seem not to play a primary role in  
virulence. This was demonstrated by A. \*\*\*salmonicida\*\*\* strains  
with deletion mutants of the GCAT and aspA genes which had no influence  
on virulence of the strains in. . .  
SUMM . . . ADP-ribosylating toxin named AexT (Aeromonas exoenzyme T)  
encoded by the gene aexT was identified in a virulent strain of A.  
\*\*\*salmonicida\*\*\* . A. \*\*\*salmonicida\*\*\* strains that were  
propagated for several passages on culture medium had lost expression of  
AexT, but still retained the aexT. . .  
SUMM [0007] Based on these observations, we used broad range gene probes to  
identify in A. \*\*\*salmonicida\*\*\* a novel type III secretion system  
by means of the gene \*\*\*acrD\*\*\* (Aeromonas calcium response D)  
encoding a transmembrane spanning protein. The \*\*\*acrD\*\*\* gene has a  
high similarity to IcrD, a protein of the Yersinia sp. which is an inner

membrane protein of the type III secretion apparatus in *Yersinia* sp. The \*\*\*acrD\*\*\* gene is flanked by further typical type III secretion genes which were designated \*\*\*acr1\*\*\*, \*\*\*acr2\*\*\*, \*\*\*acr3\*\*\*, \*\*\*acr4\*\*\*, \*\*\*acrD\*\*\*, \*\*\*acrR\*\*\*, \*\*\*acrG\*\*\*, \*\*\*acrV\*\*\*, and \*\*\*acrH\*\*\*, and which show significant similarity to pcr1, pcr2, pcr3, pcr4, pcrD, pcrR, pcrG, pcrV, and pcrH of *Pseudomonas aeruginosa* and. . . the respective bacterium, including the regulation of the low calcium response (LCR) and chaperon functions. The genes isolated from *A. salmonicida* belong to the analogue of the virA operon, which is central in the type III secretion pathway of many Gram-negative. . .

- SUMM [0008] We have also determined that the type III secretion system in *A. salmonicida* is located on a 84 kb plasmid which is rapidly lost upon growth in culture medium. Biosynthesis of \*\*\*AcrV\*\*\* in *A. salmonicida*, the analogue to LcrV in *Yersinia*, requires as a trigger either low Ca<sup>sup.2+</sup> conditions or contact with fish cells. Upon infection with *A. salmonicida* expressing \*\*\*AcrV\*\*\*, the cultured cells undergo significant morphological changes. Cultures derived from originally virulent *A. salmonicida* strains, which had lost the type III secretion genes including \*\*\*AcrV\*\*\*, lost virulence as they did not affect rainbow trout gonad cells morphologically after infection. Concomitantly to loss of the type III secretion genes, these cultures lost the expression of the aexT gene which specifies the ADP-ribosylating toxin of *A. salmonicida*.
- SUMM [0009] Rainbow trout gonad cells infected with the virulent *A. salmonicida* and incubated in antiserum directed against recombinant \*\*\*AcrV\*\*\*-His protein could be protected from the toxic effect and showed only weak morphological changes. \*\*\*AcrV\*\*\*, which belongs to the type III secretion proteins is a determinative factor involved in virulence mechanisms of *A. salmonicida*, and is expected to provide new insights into basic mechanisms of pathogenicity of bacterial species. The components of the type III secretion system of *A. salmonicida* may be used as antigens for the development of sub-unit vaccines against infection of fish by *A. salmonicida*.
- SUMM . . . invention comprises an isolated 5.7 kb nucleic acid segment (SEQ ID NO:10) containing the type III secretion genes of *A. salmonicida*. In another embodiment, the invention comprises a nucleic acid segment that encodes protein having the amino acid sequence of SEQ. . .
- SUMM [0011] In a further embodiment, the invention relates to the use of \*\*\*AcrV\*\*\* as an immunogen, and to the use of \*\*\*AcrV\*\*\* in a recombinant or traditional vaccine to reduce the incidence of infection by *A. salmonicida*.
- SUMM [0012] In another embodiment, the invention provides a means of diagnosing *A. salmonicida*, or other bacteria found to contain \*\*\*AcrV\*\*\* homologues, by the detection of the \*\*\*AcrV\*\*\* protein or the homologous proteins.
- DRWD [0013] FIG. 1 is a genetic map of the type III secretion genes found in *A. salmonicida*. Boxes with arrowheads indicate open reading frames (ORFs). The size of the different genes (in kilobases) is shown by the scale bar. A restriction map containing restriction enzymes SacI, PstI, NotI, BamHI, and SalI is shown. Abbreviation used: \*\*\*acr\*\*\*, *Aeromonas* calcium response.
- DRWD [0014] FIG. 2 is a segregation curve of *A. salmonicida* JF2267. An *A. salmonicida* JF2267 LB-culture was first incubated 21/2 hrs at 19.degree. C. and then at 22.degree. C. for 7 hrs. Colony-blotting was. . .
- DRWD [0015] FIG. 3 shows a pulsed-field gel electrophoresis of *A. salmonicida* strain JF2267, and strain JF2397. (Lane 1) JF2267, undigested. (Lane 2) JF2397, undigested. (Lane 3) JF2267 digested with NotI. (Lane. . . Low Range PFG Marker (New England Biolabs). The white arrows indicate the bands that hybridized on Southern blots with the \*\*\*acrD\*\*\* gene probe.
- DRWD [0016] FIG. 4 shows infection of fish cells with *A. salmonicida* ATCC 33658.sup.T, JF2267, and JF2397. RTG-2 cells infected with JF2267 (A), ATCC 33658.sup.T (B), JF2397 (C), and pure PBS (D). RTG-2 cells infected with JF2267 and monospecific polyclonal antibodies against \*\*\*AcrV\*\*\* were protected (E), whereas RTG-2 cells infected with JF2267 and anti-\*\*\*AcrV\*\*\* preserum were not. Pictures were taken 24 hrs after infection, respectively 21 hrs after the protection assay under a phase. . .

DRWD [0017] FIG. 5 shows low Ca.sup.2+ response induced \*\*\*AcrV\*\*\* expression in A. \*\*\*salmonicida\*\*\* JF2267. The picture shows an immunoblot reacted with specific rabbit anti- \*\*\*AcrV\*\*\* antiserum. Strains ATCC 33658.sup.T (lane 2), JF2267 (lane 3) and JF2397 (lane 4) were grown in Ca.sup.2+ depleted medium, harvested by centrifugation and analyzed on 15% SDS PAGE followed by immunoblotting. Lane 1 contains purified recombinant \*\*\*AcrV\*\*\* -His protein as a control.

DETD [0018] A 5.7 kb segment containing type III secretion genes of A. \*\*\*salmonicida\*\*\* that were cloned and sequenced correspond to the pcr locus (*Pseudomonas* calcium response) of *Pseudomonas aeruginosa* (Frank, 1997; Yahr et al. . . Gough et al., 1992; Michiels and Cornelis, 1991). The most conserved gene at this locus was revealed to be the \*\*\*acrD\*\*\* gene encoding the \*\*\*AcrD\*\*\* protein, which showed 82% identical aa to the transmembrane spanning core proteins LcrD of the injectisome of the *Y. enterocolitica*. . . *aeruginosa* type III secretion apparatus (Yahr et al., 1997b; Plano et al., 1991). Due to this high similarity, we conclude \*\*\*AcrD\*\*\* to have the analogous functions in the injectisome of the A. \*\*\*salmonicida\*\*\* type III secretion pathway.

DETD [0019] The least conserved protein encoded on the cloned and analyzed segment is \*\*\*AcrV\*\*\*, which shows only 35% identical aa to PcrV of *P. aeruginosa* and 37% identity to LcrV of *Y. enterocolitica*. The main role of LcrV and PcrV, and accordingly also of \*\*\*AcrV\*\*\*, is assumed to be involved in sensing the bacterium-host interactions (Sawa et al., 1999; Bergman et al., 1991). We therefore interpret the significantly higher dissimilarity between \*\*\*AcrV\*\*\* and LcrV or PcrV, compared to the other gene products of the type III secretion locus (Table 2), to be due to the host specificity which seems to be determined by \*\*\*AcrV\*\*\*, LcrV or PcrV.

DETD [0020] Our analyses revealed the A. \*\*\*salmonicida\*\*\* type III secretion genes to be located on a plasmid of 84 kb. The plasmid was shown to be lost. . . in particular after a slight raise in growth temperature. Concomitant to the loss of the type III genes in A. \*\*\*salmonicida\*\*\*, we detected the loss in virulence of the strain as measured by the infection of RTG-2 fish cell cultures, as. . . the loss of production of ADP-ribosylating toxin aexT in supernatants and bacterial cell pellets of low Ca.sup.2+ response induced A. \*\*\*salmonicida\*\*\* cultures. It is also noted that AexT biosynthesis induced by contact of A. \*\*\*salmonicida\*\*\* with RTG-2 fish cells disappeared in those strains or subcultures that had lost the type III secretion genes. Expression of. . .

DETD . . . III secretion mechanism was also indicated by the presence of a consensus sequence upstream the aexT toxin gene in A. \*\*\*salmonicida\*\*\*, which shows full homology to the binding site of a transcriptional activator, known in *P. aeruginosa* as ExsA, which is involved in type III dependent gene expression (Frank, 1997). The expression of aexT in A. \*\*\*salmonicida\*\*\* is thus dependent on a functional type III secretion mechanism. The lack of production of AexT as detected in the type strain of A. \*\*\*salmonicida\*\*\* ATCC 33658.sup.T as well as in the strain JF2397 which was derived from an originally virulent A. \*\*\*salmonicida\*\*\* strain, JF2267, in spite of the presence of a functional aexT gene, must therefore be due to the loss of. . .

DETD [0022] The \*\*\*AcrV\*\*\* protein of the novel type III secretion pathway of A. \*\*\*salmonicida\*\*\* plays an important role in pathogenesis by its role as a sensor and regulator of the system, as shown in. . . efficiently protected mice against challenge with *Y. pestis* (Leary et al., 1995). Our results showed that antibodies directed against recombinant \*\*\*AcrV\*\*\*, the analogous protein to LcrV, protected fish RTG-2 cells from damage caused by virulent A. \*\*\*salmonicida\*\*\* strain JF2267 and demonstrated that the \*\*\*AcrV\*\*\* plays an important role in type III secretion pathway mediated virulence of A. \*\*\*salmonicida\*\*\*.

DETD [0023] The newly found type III secretion pathway plays a central role in pathogenicity of A. \*\*\*salmonicida\*\*\* via the secretion and direct injection of the ADP-ribosylating toxin AexT into the target cells. Loss of the type III. . . due to the instability of a kb plasmid under culture conditions. Furthermore, loss of type III secretion genes such as \*\*\*acrD\*\*\* and \*\*\*acrV\*\*\* abolished expression of the aexT gene, and led to loss of virulence of A. \*\*\*salmonicida\*\*\*. As shown, surface exposed gene products of this

type III secretion pathway, in particular \*\*\*AcrV\*\*\*, are potent candidates for new vaccines for the immune prophylaxis of fish against furunculosis.

- DETD [0027] A. \*\*\*salmonicida\*\*\* strains are listed in Table 1. A. \*\*\*salmonicida\*\*\* type strain ATCC 33658.sup.T was purchased from the American Type Culture Collection. A. \*\*\*salmonicida\*\*\* strain JF2267 was freshly isolated from an arctic char (*Salvelinus alpinus*) showing typical symptoms of furunculosis. A. \*\*\*salmonicida\*\*\* strain JF2397 was derived from strain JF2267 by repeated single colony isolations after each of nine passages propagated on LB agar medium at 22.degree. C. for two days each passage. A. \*\*\*salmonicida\*\*\* strains were routinely cultured on blood agar plates (Trypticase soy agar supplemented with 0.1% CaCl<sub>2</sub> and 5% sheep blood) at . . .
- DETD [0028] Liquid cultures of A. \*\*\*salmonicida\*\*\* were made by inoculation of Trypticase soy broth (TSB) (2.75 g/100 ml Trypticase soy broth without Dextrose (BBL.RTM. 11774, Becton. . .
- DETD [0031] Genomic DNA of A. \*\*\*salmonicida\*\*\* was extracted by the guanidium hydrochloride method (Pitcher et al., 1989). A partial gene library of, A. \*\*\*salmonicida\*\*\* JF2267 was constructed by cloning agarose gel purified SacI-SalI digested fragments of 4 to 6 kb size into vector pBluescriptII-SK.sup.-. . . blot (Ausubel et al., 1999) using digoxigenin (DIG)-labeled DNA probes as described previously (Braun et al., 1999). Plasmids from A. \*\*\*salmonicida\*\*\* were purified using the method of Birnboim and Doly (Birnboim and Doly, 1979).
- DETD [0032] To construct a genomic library from A. \*\*\*salmonicida\*\*\* JF2267, 0.1 .mu.g of DNA partially digested with Sau3a was ligated to ZapExpress BamHI prepared arms (Pharmacia, Uppsala, Sweden) and. . .
- DETD . . . and NBRF databases were performed using the programs BLASTN, BLASTX and BLASTP (Altschul et al., 1990). Potentially antigenic segments of \*\*\*AcrV\*\*\* were determined using the software ProtScale (<http://www.expasy.ch/cgi-bin/protscale.pl> (Bairoch et al., 1995) and the software Coils output ([http://www.ch.embnet.org/software/COILS\\_form.html](http://www.ch.embnet.org/software/COILS_form.html)) (Lupas et al., . . .
- DETD [0039] Curing of Type III Secretion Genes from A. \*\*\*salmonicida\*\*\* :
- DETD [0040] In order to study the segregation of the type III secretion genes in A. \*\*\*salmonicida\*\*\* strain JF2267, the strain was inoculated in LB-broth at a density of A.sub.600=0.08 and incubated 21/2 hrs at 19.degree. C. . .
- DETD [0042] The bacterial strains A. \*\*\*salmonicida\*\*\* JF 2267 and JF2397 were grown on LB agar for one day at room temperature. Then bacterial suspensions in 10. . .
- DETD [0046] Expression and Purification of His-Tailed Fusion Protein \*\*\*AcrV\*\*\* :
- DETD [0047] Oligonucleotide primers used to amplify the whole \*\*\*acrV\*\*\* gene are given in Table 2. The PCR reactions were carried out as described above with the exception of using. . . DNA polymerase (Expand Long Template PCR System kit, Roche Diagnostics) instead of Taq DNA polymerase and genomic DNA of A. \*\*\*salmonicida\*\*\* JF2267. The PCR products were purified by using the High Pure PCR Product Purification Kit (Roche Diagnostics) as described by the manufacturer's protocol. Then the \*\*\*acrV\*\*\* PCR product was cloned into pGEM-T vector (Promega, Madison, Wis. USA), having 3'-T overhangs at the insertion sites, as described. . .
- DETD [0048] Production of Monospecific Rabbit Anti- \*\*\*AcrV\*\*\* Antibodies and Immunoblot Analyses:
- DETD [0049] Monospecific, polyclonal antibodies directed against \*\*\*AcrV\*\*\* were obtained by immunizing rabbits subcutaneous with 80 .mu.g of recombinant polyhistidine-tailed \*\*\*AcrV\*\*\* protein in 200 .mu.l PN buffer and 150 .mu.l NaCl (0.85%) mixed with 350 .mu.l Freund's complete adjuvant (Difco Laboratories, . . .
- DETD [0050] Infection of Fish Cell Cultures with A. \*\*\*salmonicida\*\*\* :
- DETD . . . and 4 mio cells were seeded into a 25 cm.sup.2 tissue culture flask. Monolayered RTG-2 cells were infected with A. \*\*\*salmonicida\*\*\* cells resuspended in phosphate buffered saline (PBS) pH 7.4 at a multiplicity of infection of 20:1 or 2:1 (bacteria/fish cells).. . .
- DETD [0052] Protection Assay Using Rabbit Antiserum \*\*\*AcrV\*\*\* :
- DETD . . . cells were seeded into 24 well culture plates (1.9 cm.sup.2) (Techno plastic products AG, Trasadingen, Switzerland). Rabbit antiserum directed against \*\*\*AcrV\*\*\* as well as control preserum were decomplexed for 30 min at 56.degree. C. A fresh culture of A. \*\*\*salmonicida\*\*\* (at end exponential growth phase) was washed and

resuspended in PBS pH 7.4 and mixed with either preserum or anti  
 \*\*\*AcrV\*\*\* antiserum at a ratio of 1:1, 1:10, 1:100, 1:1000 or  
 1:10,000. Bacteria were incubated with the serum at 18.degree. C. . . .  
 DETD . . . immunoblotting, Western-blot were blocked with 1% milk buffer  
 for at least one hour and then incubated with the rabbit antiserum  
 \*\*\*AcrV\*\*\* (1:2000) or with the rabbit preserum (1:1000) in milk  
 buffer overnight at 4.degree. C. The membranes were then washed  
 thoroughly. . . .  
 DETD [0056] Cloning and Sequence Analysis of the virA Locus of a Type III  
 Pathway of A. \*\*\*salmonicida\*\*\* :  
 DETD [0057] Analysis of A. \*\*\*salmonicida\*\*\* strain JF2267 with an array  
 of broad range probes for detection of type III secretion pathways  
 revealed a strong signal. . . . type III secretion pathway of Yersinia  
 pestis and Pseudomonas aeruginosa. In analogy to the Y. pestis genes, we  
 named them \*\*\*acr1\*\*\*, \*\*\*acr2\*\*\*, \*\*\*acr3\*\*\*, \*\*\*acr4\*\*\*  
 , and \*\*\*acrD\*\*\* (Aeromonas calcium response (FIG. 1)). They are  
 located on a single operon followed by a transcription termination  
 signal similar to. . . . 1996; Iriarte and Cornelis, 1999; Plano et  
 al., 1991; Cornelis, 1998; Yahr et al., 1997a). The similarities of the  
 genes \*\*\*acr1\*\*\*, \*\*\*acr2\*\*\*, \*\*\*acr3\*\*\*, \*\*\*acr4\*\*\* and  
 \*\*\*acrD\*\*\* with the analogues in Y. enterocolitica and in P. aeruginosa  
 are given in Table 2. Downstream lcrD we identified a locus with a  
 canonical promoter sequence followed by further genes named \*\*\*acrR\*\*\*  
 , \*\*\*acrG\*\*\*, and \*\*\*acrV\*\*\* on a separate operon (FIG. 1)  
 according to the corresponding genes in Y. pestis (Table 3) (Barve and  
 Straley, 1990; Skrzypek and Straley, 1993; Nilles et al., 1998). The ORF  
 of the putative \*\*\*acrV\*\*\* gene seemed to be incomplete on the 4.8  
 kb SacI-SalI fragment of pJFFIVB638, and represented only the 5'-half of  
 the gene. The remaining part of \*\*\*acrV\*\*\* and part of \*\*\*acrH\*\*\*  
 located downstream of \*\*\*acrV\*\*\* were cloned separately from the  
 .lambda. phage gene library of A. \*\*\*salmonicida\*\*\* as an  
 overlapping clone which was obtained by screening the gene library using  
 a gene probe for the 5'-half of \*\*\*acrV\*\*\* which was produced by PCR  
 with primers \*\*\*AcrV\*\*\* -L and \*\*\*AcrV\*\*\* -R (Table 2). The  
 resulting plasmid based on vector pBK-CMV was designated pJFFIVB832.  
 From this plasmid, a 0.9 kb SalI fragment containing the 3' end of  
 \*\*\*acrV\*\*\* and part of the downstream gene \*\*\*acrH\*\*\* was  
 subcloned on pBluescriptII-SK and designated pJFFIVB828.  
 DETD [0058] Instability of the Genes Belonging to the Type III Pathway in A.  
 \*\*\*salmonicida\*\*\* :  
 DETD [0059] When we analyzed the different A. \*\*\*salmonicida\*\*\* strains  
 with a specific probe for \*\*\*acrD\*\*\*, we discovered by using  
 Southern blot hybridization that the \*\*\*acrD\*\*\* gene was present  
 only in strain JF2267 but not in the derivative strain JF2397 which had  
 undergone nine passages of subsequent single colony cloning isolation.  
 Additionally, the type strain of A. \*\*\*salmonicida\*\*\*, ATCC  
 33658.sup.T, did not show a signal with the \*\*\*acrD\*\*\* probe.  
 However, several A. \*\*\*salmonicida\*\*\* strains that were freshly  
 isolated from salmon and trout with furunculoses did contain  
 \*\*\*acrD\*\*\* (Table 4). These results indicate that the type III  
 secretion pathway of A. \*\*\*salmonicida\*\*\* may be lost easily. In  
 order to get an estimate on the loss of the type III secretion genes, we  
 have analyzed the kinetics of disappearance of \*\*\*acrD\*\*\* after a  
 shift of growth temperature of strain JF2267 from 19.degree. C. to  
 22.degree. C. Colony hybridization with the \*\*\*acrV\*\*\* probe  
 revealed that in a fresh culture of strain JF2267, the \*\*\*acrD\*\*\*  
 gene was present in all cells grown at 19.degree. C. After the shift to  
 22.degree. C., \*\*\*acrD\*\*\* was still present for further 5 1/2 hrs,  
 following which it was lost very rapidly within less than 1 hr (FIG. 2).  
 Taking into account the generation time of 2 h for A.  
 \*\*\*salmonicida\*\*\* under the given growth conditions, the \*\*\*acrD\*\*\*  
 gene was lost within two generations. To analyze the loss of  
 \*\*\*acrD\*\*\* further, undigested and NotI digested genomic DNA of A.  
 \*\*\*salmonicida\*\*\* strain JF2267 and of the \*\*\*acrD\*\*\* deficient  
 derivative strain JF2397 were submitted to pulse field gel  
 electrophoresis (PFGE) and subsequent Southern blot hybridization with  
 the \*\*\*acrD\*\*\* probe. PFGE analyses of total undigested DNA revealed  
 the presence of two large plasmids in strain JF2267 while in strain . . .  
 . compared to JF2267 as the sole detectable difference (FIG. 3).  
 Southern-blot hybridization of the DNA on this gels with the  
 \*\*\*acrD\*\*\* probe confirmed the larger plasmid and the 84 kb NotI

fragment of strain JF 2267 to contain \*\*\*acrD\*\*\* gene. Neither the remaining large plasmid in JF2397 nor any of its NotI fragments hybridized with the \*\*\*AcrV\*\*\* probe. This indicates that the type III secretion genes, or at least the virA operon thereof, are located on a. . .

- DETD [0060] Presence of \*\*\*acrD\*\*\* in A. \*\*\*salmonicida\*\*\* Strains:
- DETD [0061] In order to assess the presence of the \*\*\*acrD\*\*\* gene in various A. \*\*\*salmonicida\*\*\* strains, DNA samples extracted from A. \*\*\*salmonicida\*\*\* Type strain ATCC33658 and various field strains isolated from salmon or char were digested with restriction enzymes Sall and SacI, separated by 0.7% agarose gel electrophoresis, blotted onto nylon membranes and hybridized with the \*\*\*acrD\*\*\* gene probe. The Southern blot revealed the presense of the \*\*\*acrD\*\*\* gene on a 4.8 kb fragment in all strains except in the type strain ATCC33658, the laboratory strain JF2396 which was used for the type III secretion genes, and A. \*\*\*salmonicida\*\*\* strain MT44 known to be a virulent for trout. One field strain, # 24, showed a very weak hybridization signal indicating that the culture contains \*\*\*acrD\*\*\* only in a minor population of the cells (Table 1).
- DETD [0062] Infection of RTG-2 Fish Cells and Protection of Cell Damage with Anti- \*\*\*AcrV\*\*\* Antiserum:
- DETD [0063] Freshly cultured A. \*\*\*salmonicida\*\*\* strain JF2267 was used to infect RTG-2 cells. After 24 hrs of incubation the fish cells were rounded up and also detached from the plastic support (FIG. 4A). In contrast cells infected with A. \*\*\*salmonicida\*\*\* type strain ATCC 33658.sup.T or strain JF2397 (FIGS. 4B and C), both known to be devoid of \*\*\*acrD\*\*\* and \*\*\*acrV\*\*\*, showed no morphological changes at all in spite of a massive multiplication of the bacteria in the cultures. RTG-2 fish cells which were incubated with PBS buffer as control showed no morphological changes like the cells infected with the \*\*\*acrD\*\*\* and \*\*\*acrV\*\*\* deficient strains JF2397 or ATCC 33658.sup.T (FIG. 4D).
- DETD [0064] In order to study further the role of the newly detected type III secretion pathway in virulence of A. \*\*\*salmonicida\*\*\*, we incubated strain JF 2267 with monospecific polyclonal anti- \*\*\*AcrV\*\*\* antibodies prior to infection of RTG-2 fish cell cultures. When RTG-2 fish cells were infected with strain JF2267 that was incubated with rabbit anti- \*\*\*AcrV\*\*\* antibodies diluted 1:1 or 1:10, the characteristic morphological changes of the cells were reduced, significantly affecting only 20% of the. . . with JF 2267 that was pretreated with serum from the same rabbit taken before immunization (FIG. 4F). Titration of the anti- \*\*\*AcrV\*\*\* serum showed that protection of about 50% of the RTG-2 cells could still be reached with a serum dilution of. . .
- DETD [0065] Expression of \*\*\*AcrV\*\*\* in A. \*\*\*salmonicida\*\*\* :
- DETD [0066] The expression of \*\*\*AcrV\*\*\* in A. \*\*\*salmonicida\*\*\* strain JF2267 was assessed by immunoblots using \*\*\*AcrV\*\*\* -His antibodies. When A. \*\*\*salmonicida\*\*\* was grown under standard culture conditions in TSB medium, no \*\*\*AcrV\*\*\* protein could be detected from total cells nor from culture supernatant of strain JF 2267, nor in the control of. . . Ca.sup.2+ response by chelating free Ca.sup.2+ ions in the growth medium by the addition of 10 mM NTA, we detected \*\*\*AcrV\*\*\* with anti- \*\*\*AcrV\*\*\* antibodies in the pellet of JF2267 as a protein of about 37 kDa (FIG. 5) but not in strains JF2397 and ATCC33658.sup.T, which are both devoid of the \*\*\*AcrV\*\*\* gene (FIG. 5). No \*\*\*AcrV\*\*\* protein could be detected in the supernatants of cultures from strains JF2267, JF2396 and ATCC33658.sup.T, grown in Ca.sup.2+ depleted medium.
- DETD . . . Ca.sup.2+ ions) and then put in contact with RTG-2 cells at a ratio 2:1 (bacteria: cells) for 30 minutes, the \*\*\*AcrV\*\*\* protein. could be monitored on immunoblots reacting with anti- \*\*\*AcrV\*\*\*, similar to cultures from Ca.sup.2+ depleted medium.
- DETD [0068] Recombinant \*\*\*AcrV\*\*\* Vaccine Trial
- DETD . . . M., Kay, W. W. and Trust, T. J.: Structure of the tetragonal surface virulence array protein and gene of Aeromonas \*\*\*salmonicida\*\*\*. J.Biol.Chem. 266 (1991) 15258-15265.
- DETD [0091] Hirono, I. and Aoki, T.: Cloning and characterization of three hemolysin genes from Aeromonas \*\*\*salmonicida\*\*\*. Microb.Pathog. 15 (1993) 269-282.
- DETD . . . K. and Ellis, A. E.: Glycerophospholipid:cholesterol acyltransferase complexed with lipopolysaccharide (LPS) is a major

lethal exotoxin and cytotoxin of *Aeromonas* \*\*\*salmonicida\*\*\* : LPS stabilizes and enhances toxicity of the enzyme. J.Bacteriol. 172 (1990) 5382-5393.

- DETD [0112] Thornton, J. C., Garduno, R. A., Carlos, S. J. and Kay, W. W.: Novel antigens expressed by *Aeromonas* \*\*\*salmonicida\*\*\* grown in vivo. Infect.Immun. 61 (1993) 4582-4589.
- DETD [0113] Titball, R. W. and Munn, C. B.: The purification and some properties of H-lysin from *Aeromonas* \*\*\*salmonicida\*\*\*. J.Gen.Microbiol. 131 (1985) 1603-1609.
- DETD . . . P. W., Landon, M. and Coleman, G.: The cloning and nucleotide sequence of the serine protease gene (aspA) of *Aeromonas* \*\*\*salmonicida\*\*\* ssp. \*\*\*salmonicida\*\*\*. FEMS Microbiol.Lett. 78 (1992) 65-71.
- DETD [0118] Recombinant \*\*\*AcrV\*\*\* Vaccine Trial
- DETD [0121] 1. The \*\*\*AcrV\*\*\* vaccine was formulated using recombinant, Histidine-tagged \*\*\*AcrV\*\*\* resuspended in 10 mM phosphate buffer, pH 7.0, to 112.5 .mu.g/mL. Four parts of this protein solution were mixed with one part oil adjuvant for a final \*\*\*AcrV\*\*\* concentration of 90 .mu.g/mL. The dose for testing was 0.1 mL, or 9 .mu.g/fish.
- DETD [0128] At least 50 fish are vaccinated 0.1 mL of \*\*\*AcrV\*\*\* vaccine via intra-peritoneal (IP) injection, or 0.2 mL of the commercial vaccine MultiVacc4. At the same time a group of . . .
- DETD . . . 350-degree days post vaccination 50 fish per group were challenged by IP injection with a pre-determined concentration of virulent *Aeromonas* \*\*\*salmonicida\*\*\*. The dosage depends on the source of the fish and the water temperature (this is determined empirically immediately prior to. . .
- DETD [0133] Results

	Group	% Mortality	RPS
	PBS	82	--
	***AcrV***		49 40
	MultiVacc4	30	63
DETD	[0134] 1. There was a strong challenge with 82% control mortalities.		

TABLE 1

A. \*\*\*salmonicida\*\*\* strains used in this study and presence of \*\*\*acrD\*\*\*

strain	origin	***acrD***
.sup.a)		
ATCC33658	American Type Culture Collection, Type strain	-
JF2267	Char ( <i>Salvelinus alpinus</i> ), Switzerland	+
JF2396	Laboratory strain, derivative of JF2267	-
CC-23	Salmon, Norway. . . blot hybridization	
.sup.b)	very weak hybridization signal indicating that only a minor part of the population of the culture contains the ***acrD*** gene	

DETD . . .

TABLE 2

#### Oligonucleotide primers

##### Annealing

Name	Sequence.sup.a 5' to 3'	Position.sup.b
	temp. .degree. C.	
AslcrD-L.sup.c	GCCCGTTTTGCCTATCAA	1159-1176 60
AslcrD-R.sup.c	GCGCCGATATCGGTACCC	2028-2011 60
***AcrV*** 58	-L.sup.c TTCGTCGGCTGGCTTGATGT	4144-4163
***AcrV*** 58	-R.sup.c GAACTCGCCCCCTTCCATAA	4734-4715
AsacrVt-L.sup.d	gggaattcGATGAGCACAAATCCCTGACTAC	4104-4125 57



AsacrVt-Rd atgcgggccgcAAATGCGCCAAGAATGTCG 5188-5169 57  
 AsacrVN'-R.sup.d tcgcgggccgcACCCTTTACGCTGATTGTC 4555-4537 57  
 AsacrVC'-L.sup.d cggaattcGTTGCGGGATGAGCTGGCAG 4554-4573 57  
 AsacrVC'-R.sup.d. . . 57

.sup.aLowercase letters indicate nucleotides added to create restriction enzyme  
 recognition sites (underlined) for cloning.  
 .sup.bBased on nucleotide sequence of A. \*\*\*salmonicida\*\*\* JF2267  
 .sup.cPrimer used for gene probe preparation  
 .sup.dPrimer used for amplification of gene \*\*\*acrV\*\*\* , \*\*\*acrV\*\*\* -N,  
 and \*\*\*acrV\*\*\* -C respectively

DETD [0136]  
 TABLE 3

A. \*\*\*salmonicida\*\*\* type III proteins compared to analogues In P.  
 aeruginosa and in V. enterocolitica.

Protein in Analogue in Similarlty/ Genbank Analogue in  
 Similarity/ Genbank

A. \*\*\*salmonicida\*\*\* P. aeruginosa identity.sup.a) access. nr. Y.  
 enterocolitica Identity.sup.a) access. nr. Proposed function

***Acr1***	Pcr1	80/60	AF019150	TyeA
83/69	AF102990	part of the translocation-control apparatus, required for selective translocation of Yops		
***Acr2***	Pcr2	63/44	AF019150	SycN
77/62	AF102990	chaperone forYopN		
***Acr3***	Pcr3	62/47	AF019150	YscX
69/54	AF102990	part of the type III secretion apparatus, secretion of Yop		
***Acr4***	Pcr4	66/55	AF019150	YscY
64/52	AP102990	part of the type III secretion apparatus, secretion of Yop		
***AcrD***	PcrD	90/82	AF019150	LcrD
90/82	X87771	inner membrane spanning protein of type III secretion		
***AcrR***	PcrR	68/58	AF019150	LcrR
71/58	AF102990			
***AcrG***	PcrG	63/46	AF010149	LcrG
64/42	AF102990	regulation of low calcium response		
***AcrV***	PcrV	50/35	AF010149	LcrV
53/37	X96797	regulation of low calcium response, sensor suppression of TNFa and Interferon a, protective antigen		
***AcrH***	PcrH	78/65	AF010149	LcrH (SycD)
79/58	AF102990	regulation ot low calcium response, chaperon for YopD, secretion		

.sup.a)given as % of similar/identical. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 2

LENGTH: 123

TYPE: PRT

ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*

SEQUENCE: 2

Met Asn Trp Ile Glu Pro Leu Leu Val Gln Phe Cys Gln Asp Leu Gly

1 5 10. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 3

LENGTH: 121

TYPE: PRT

ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*

SEQUENCE: 3

Met Ser Arg Ile Thr Ala Ala His Ile Gly Ile Glu Gln Leu Ser Ala

1 5 10. . .

DETD SEQUENCE CHARACTERISTICS:

SEQ ID NO: 4

LENGTH: 116  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 4  
 Met Thr Met Val Leu Thr Ser Gln Gln Gln Asp Ala Leu Leu Leu Thr  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 5  
 LENGTH: 705  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 5  
 Met Asn Gln Arg Thr Leu Glu Leu Leu Arg Arg Ile Gly Glu Arg Lys  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 6  
 LENGTH: 93  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 6  
 Met Leu Val Arg Arg Glu Gly Glu Arg Ala Gly Leu Ala Asn Pro Phe  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 7  
 LENGTH: 94  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 7  
 Met Lys Gln Pro Arg Phe Ala Asp His Ser Glu Thr Ile Ser Gln Ala  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 8  
 LENGTH: 361  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 8  
 Met Ser Thr Ile Pro Asp Tyr Asn Thr Asn Pro Gly Ala Phe Val Gly  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 9  
 LENGTH: 159  
 TYPE: PRT  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 9  
 Met Gln Thr Asp Thr Thr Leu Thr Pro Glu Tyr Glu Ala Glu Leu Glu  
 1 5 10. . .  
 DETD SEQUENCE CHARACTERISTICS:  
 SEQ ID NO: 10  
 LENGTH: 5678  
 TYPE: DNA  
 ORGANISM: Aeromonas \*\*\*salmonicida\*\*\*  
 SEQUENCE: 10  
 gagctcaagc ggctgatccg cctgctgccg gtggagctgt tcagtgaaga ggagcagcgc 60  
 cagaatctgt tgcagtgtcg tcagggtgcg ctcgataacg ccatcgagcg ggaagaggat 120  
 gagttgtctg gagagtcgtc atgaactgga ttgaaccctc gctggtgcag. . .

CLM What is claimed is:

23. An isolated polypeptide comprising at least one epitope or epitopic region of a selected one of the class \*\*\*Acr1\*\*\* ; \*\*\*Acr2\*\*\* ; \*\*\*Acr3\*\*\* ; \*\*\*Acr4\*\*\* ; \*\*\*AcrD\*\*\* ; \*\*\*AcrR\*\*\* ; \*\*\*AcrG\*\*\* ; \*\*\*AcrV\*\*\* ; and \*\*\*AcrH\*\*\* .

29. A method for reducing the susceptibility of fish to infection by a virulent strain of A. \*\*\*salmonicida\*\*\* comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of a composition comprising an immunogenic amount of at least one epitope or epitopic region of \*\*\*AcrV\*\*\* , any other protein of the A.

\*\*\*salmonicida\*\*\* Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof.

- . . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. \*\*\*salmonicida\*\*\* Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .
- . . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. \*\*\*salmonicida\*\*\* Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .
- . . . The method of claim 29, wherein the at least one epitope or epitopic region of a protein of the A. \*\*\*salmonicida\*\*\* Type III secretion apparatus, a natural or genetically modified variant thereof, or an antigenic peptide derived or synthesized thereof, is. . .
33. A method for reducing the susceptibility of fish to infection by a virulent strain of A. \*\*\*salmonicida\*\*\* comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of an immunogenic amount of a composition comprising the \*\*\*acrV\*\*\* gene, the gene of any other protein of the A. \*\*\*salmonicida\*\*\* Type III secretion apparatus, homologues, fragments, or synthetic oligonucleotides derived thereof.
34. A method for reducing the susceptibility of fish to infection by a virulent strain of A. \*\*\*salmonicida\*\*\* comprising the intraperitoneal, intramuscular, intradermal, intracellular, spray, immersion, or oral administration to said fish of an immunogenic amount of a. . .
35. A therapeutic method for the protection of fish from the toxic effect of a virulent strain of A. \*\*\*salmonicida\*\*\* comprising the use of antiserum directed against \*\*\*AcrV\*\*\*, variants or fragments thereof, or synthesized peptides thereof.
36. The method of claim 35 wherein the antiserum is directed against recombinant \*\*\*AcrV\*\*\*.

L14 ANSWER 5 OF 27 USPTAFULL on STN

AN 2004:7465 USPTAFULL

TI Poroplasts

IN Surber, Mark W., Coronado, CA, UNITED STATES  
Giacalone, Matthew, San Diego, CA, UNITED STATES

PI US 2004005700 A1 20040108

AI US 2002-157339 A1 20020528 (10)

DT Utility

FS APPLICATION

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CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18539

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DRWD [0313] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to

\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DRWD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DRWD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing

in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhvRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);  
angR gene. . . .

DRWD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene  
(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic  
Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J.  
Jorgensen, and I. van. . . . 8:2703-2709); the secE gene (Muller, M.,  
et al. 1002. Protein traffic in bacteria: multiple routes from the  
ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res.  
Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002.  
Protein traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
66:107-157).

DRWD . . . that is in contact with the minicell composition. By  
"semipermeable" it is meant that certain agents can be freely exchanged  
\*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
the membrane or the other. For example, the toxic compound. . . .

DRWD [0471] Many Gram-negative pathogens use a type III secretion machine to  
translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
(for a review, see Cheng L W, Schneewind O. Type III machines of  
Gram-negative bacteria: delivering. . . .

DRWD . . . a database devoted to the ATP-binding cassette (ABC) protein  
domains (ABCDdb), the majority of which energize the transport of  
compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
involved in the uptake of a wide range of molecules and in mechanisms  
of. . . .

DRWD [0581] A "channel protein" is a protein that facilitates the diffusion  
of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
hydrophilic pore or "channel" that provides molecules/ions access  
through lipid membranes, which are generally hydrophobic. . . .

DRWD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
blood-brain barrier to the brain. This modality is used, by way of  
non-limiting example, for imaging purposes, and for. . . .

DRWD . . . polymers include acidic gelatin, polygalactosamine, polyamino  
acids such as polylysine, polyhistidine, polyornithine, polyquaternary  
compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE),  
DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran,  
DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates,  
polyamidoamines, cationic starches, polyvinylpyridine, and  
polythiodiethylaminomethylethylene.

L14 ANSWER 6 OF 27 USPATFULL on STN

AN 2003:330124 USPATFULL

TI Minicell-based screening for compounds and proteins that modulate the  
activity of signalling proteins

IN Surber, Mark W., Coronado, CA, UNITED STATES  
Berkley, Neil, San Diego, CA, UNITED STATES

PI US 2003232335 A1 20031218

AI US 2002-157317 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18564

AB The invention provides compositions and methods for the production of  
achromosomal and anucleate cells useful for applications such as  
diagnostic and therapeutic uses, as well as research tools and agents  
for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and

expression factors from *E. coli* include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
 plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
 traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
 bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
 membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
 (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
 from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic  
 Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
 Jorgensen, and l.. . . 8:2703-2709); the secE gene (Muller, M., et  
 al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
 to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
 in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
 the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).  
 DETD . . . that is in contact with the minicell composition. By  
 "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .  
 DETD [0466] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .  
 DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCdb), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .  
 DETD [0575] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access  
 through lipid membranes, which are generally hydrophobic.. . .  
 DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
 also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
 blood-brain barrier to the brain. This modality is used, by way of  
 non-limiting example, for imaging purposes, and for. . .  
 DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino  
 acids such as polylysine, polyhistidine, polyornithine" polyquaternary  
 compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE),  
 DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran,  
 DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates,  
 polyamidoamines, cationic starches, polyvinylpyridine, and  
 polythiodiethylaminomethylethylene.

L14 ANSWER 7 OF 27 USPTAFULL on STN  
 AN 2003:318700 USPTAFULL  
 TI Antibodies to native conformations of membrane proteins  
 IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
 Berkley, Neil, San Diego, CA, UNITED STATES  
 Surber, Mark W., Coronado, CA, UNITED STATES  
 PI US 2003224444 A1 20031204  
 AI US 2002-157491 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18559

AB The invention provides compositions and methods for the production of  
achromosomal and anucleate cells useful for applications such as  
diagnostic and therapeutic uses, as well as research tools and agents  
for drug discovery.

DETD [0313] Regulatory elements, promoters and other expression elements and  
expression factors from *E. coli* include but are not limited to  
\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic  
Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M.,  
et al. 1002. Protein traffic in bacteria: multiple routes from the  
ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res.*  
*Mol. Biol.* 66:107-157); and the secY gene (Muller, M., et al. 1002.  
Protein traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157).

DETD . . . that is in contact with the minicell composition. By  
"semipermeable" it is meant that certain agents can be freely exchanged  
\*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
the membrane or the other. For example, the toxic compound. . .

DETD [0464] Many Gram-negative pathogens use a type III secretion machine to  
translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
(for a review, see Cheng L W, Schneewind O. Type III machines of  
Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
domains (ABCDdb), the majority of which energize the transport of  
compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
involved in the uptake of a wide range of molecules and in mechanisms  
of. . .

DETD [0569] A "channel protein" is a protein that facilitates the diffusion  
of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
hydrophilic pore or "channel" that provides molecules/ions access  
through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
blood-brain barrier to the brain. This modality is used, by way of  
non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino

acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copoly methacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 8 OF 27 USPATFULL on STN

AN 2003:318625 USPATFULL

TI Reverse screening and target identification with minicells

IN Surber, Mark W., Coronado, CA, UNITED STATES

Berkley, Neil, San Diego, CA, UNITED STATES

Gerhart, William, La Mesa, CA, UNITED STATES

PI US 2003224369 A1 20031204

AI US 2002-157171 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18610

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to

\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); *ampD* (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the *LuxRI* homologs *AhyRI* and *AsaRI* and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the *LuxRI* homologs *AhyRI* and *AsaRI* and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . 175:5009-5021); the *ffh* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the *FtsY* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the *fimC* gene (Klemm P., and B. J. Jorgensen, and l.. . . 8:2703-2709); the *secE* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); and the *secY* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0467] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDh), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0576] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 9 OF 27 USPATFULL on STN

AN 2003:312291 USPATFULL

TI Minicell-based bioremediation

IN Segall, Anca M., San Diego, CA, UNITED STATES

Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003219888 A1 20031127

AI US 2002-157418 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)

US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18632

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0347] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to

\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); *ampD* (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\**: identification of the *LuxRI* homologs *AhyRI* and *AsaRI* and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\**: identification of the *LuxRI* homologs *AhyRI* and *AsaRI* and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . 175:5009-5021); the *ffh* gene (Muller, M., et al. 1997. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1997. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the *FtsY* gene (Muller, M., et al. 1997. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic*



Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0488] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng LW, Schneewind O. Type III machines of Gram-negative bacteria: delivering the. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0581] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 10 OF 27 USPATFULL on STN

AN 2003:311814 USPATFULL

TI Methods of making pharmaceutical compositions with minicells

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES

Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003219408 A1 20031127

AI US 2002-157320 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)

US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18632

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);

angR gene. . . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and l. . . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymerethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 11 OF 27 USPATFULL on STN

AN 2003:300375 USPATFULL

TI Minicell-based delivery agents

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Klepper, Robert, San Diego, CA, UNITED STATES  
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003211599 A1 20031113

AI US 2002-157106 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)  
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18671

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); *ampD* (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** : identification of the LuxRI homologs *AhyRI* and *AsaRI* and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation *ahyRI* gene product from *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas \*\*\*salmonicida\*\*\** : identification of the LuxRI homologs *AhyRI* and *AsaRI* and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); *angR* gene. . .

DETD . . . 175:5009-5021); the *ffh* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the *FtsY* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the *fimC* gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the *secE* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); and the *secY* gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\* , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

IN Berkley, Neil, San Diego, CA, UNITED STATES  
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003211086 A1 20031113  
AI US 2002-157073 A1 20020528 (10)  
PRAI US 2001-295566P 20010605 (60)  
US 2002-359843P 20020225 (60)

DT Utility  
FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 17  
ECL Exemplary Claim: 1  
DRWN 2 Drawing Page(s)  
LN.CNT 18553

AB The invention provides compositions and methods for the production of  
achromosomal and anucleate cells useful for applications such as  
diagnostic and therapeutic uses, as well as research tools and agents  
for drug discovery.

DETD [0313] Regulatory elements, promoters and other expression elements and  
expression factors from *E. coli* include but are not limited to  
\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157); 4.5 S RNA (Muller, M., et al. 1002. Protein traffic in  
bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic  
Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
Jorgensen, and I. . . 8:2703-2709); the secE gene (Muller, M., et  
al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By  
"semipermeable" it is meant that certain agents can be freely exchanged  
\*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
the membrane or the other. For example, the toxic compound. . .

DETD [0470] Many Gram-negative pathogens use a type III secretion machine to  
translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
(for a review, see Cheng L W, Schneewind O. Type III machines of  
Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
domains (ABCDdb), the majority of which energize the transport of  
compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
involved in the uptake of a wide range of molecules and in mechanisms  
of. . .

DETD [0578] A "channel protein" is a protein that facilitates the diffusion  
of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
hydrophilic pore or "channel" that provides molecules/ions access  
through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can

also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 13 OF 27 USPATFULL on STN

AN 2003:294815 USPATFULL

TI Pharmaceutical compositions with minicells

IN Berkley, Neil, San Diego, CA, UNITED STATES  
Klepper, Robert, San Diego, CA, UNITED STATES  
Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003207833 A1 20031106

AI US 2002-156811 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18585

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0468] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . . .

DETD [0577] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 14 OF 27 USPTAFULL on STN

AN 2003:288723 USPTAFULL

TI Conjugated minicells

IN Surber, Mark W., Coronado, CA, UNITED STATES  
Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003203481 A1 20031030

AI US 2002-157213 A1 20020528 (10)

PRAI US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 12

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18551

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene

(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0467] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0576] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 15 OF 27 USPATFULL on STN

AN 2003:288653 USPATFULL

TI Methods of minicell-based delivery

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Berkley, Neil, San Diego, CA, UNITED STATES  
Klepper, Robert, San Diego, CA, UNITED STATES  
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003203411 A1 20031030

AI US 2002-156792 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)  
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18582

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* :

identification of the LuxRI homologs AhvRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahvRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhvRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 16 OF 27 USPATFULL on STN

AN 2003:288179 USPATFULL

TI Minicell-based diagnostics

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES

Klepper, Robert, San Diego, CA, UNITED STATES

Berkley, Neil, San Diego, CA, UNITED STATES

PI US 2003202937 A1 20031030

AI US 2002-157178 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)

US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 19

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)



LN.CNT 18527

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0314] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0471] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCDb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0580] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\* , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 17 OF 27 USPATFULL on STN

AN 2003:282746 USPATFULL  
 TI Membrane to membrane delivery  
 IN Surber, Mark W., Coronado, CA, UNITED STATES  
 Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
 PI US 2003199089 A1 20031023  
 AI US 2002-157318 A1 20020528 (10)  
 PRAI US 2001-295566P 20010605 (60)  
 US 2002-359843P 20020225 (60)  
 DT Utility  
 FS APPLICATION  
 LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
 IRVINE, CA, 92614  
 CLMN Number of Claims: 20  
 ECL Exemplary Claim: 1  
 DRWN 2 Drawing Page(s)  
 LN.CNT 18530  
 AB The invention provides compositions and methods for the production of  
 achromosomal and anucleate cells useful for applications such as  
 diagnostic and therapeutic uses, as well as research tools and agents  
 for drug discovery.  
 DETD [0346] Regulatory elements, promoters and other expression elements and  
 expression factors from *E. coli* include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
 plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
 traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
 bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
 membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
 (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
 from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic*  
*Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
 Jorgensen, and 1. . . 8:2703-2709); the secE gene (Muller, M., et  
 al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
 to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
 in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
 the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).  
 DETD . . . that is in contact with the minicell composition. By  
 "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .  
 DETD [0503] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .  
 DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCdb), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .  
 DETD [0612] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access

through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 18 OF 27 USPATFULL on STN

AN 2003:282745 USPATFULL

TI Minicell-based gene therapy

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Berkley, Neil, San Diego, CA, UNITED STATES  
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003199088 A1 20031023

AI US 2002-156902 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)  
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 15300

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to  
\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and L. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By

"semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .

DETD [0502] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCDdb), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .

DETD [0611] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access  
 through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
 also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
 blood-brain barrier to the brain. This modality is used, by way of  
 non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino  
 acids such as polylysine, polyhistidine, polyornithine" polyquaternary  
 compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE),  
 DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran,  
 DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates,  
 polyamidoamines, cationic starches, polyvinylpyridine, and  
 polythiodiethylaminomethylethylene.

L14 ANSWER 19 OF 27 USPTAFULL on STN

AN 2003:282662 USPTAFULL

TI Solid supports with minicells

IN Sabbadini, Roger, Lakeside, CA, UNITED STATES  
 Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003199005 A1 20031023

AI US 2002-157166 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)  
 US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
 IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18494

AB The invention provides compositions and methods for the production of  
 achromosomal and anucleate cells useful for applications such as  
 diagnostic and therapeutic uses, as well as research tools and agents  
 for drug discovery.

DETD [0312] Regulatory elements, promoters and other expression elements and  
 expression factors from E. coli include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
 plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
 Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include  
 without limitation ahyRI gene product from Aeromonas hydrophila and  
 Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* ;  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);  
 angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include  
 without limitation ahyRI gene product from Aeromonas hydrophila and  
 Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* ;  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);  
 angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein

traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
 bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
 membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene  
 (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
 from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic  
 Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J.  
 Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et  
 al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
 to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
 in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
 the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By  
 "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .

DETD [0469] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCD), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .

DETD [0578] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access  
 through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
 also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
 blood-brain barrier to the brain. This modality is used, by way of  
 non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino  
 acids such as polylysine, polyhistidine, polyornithine" polyquaternary  
 compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE),  
 DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran,  
 DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates,  
 polyamidoamines, cationic starches, polyvinylpyridine, and  
 polythiodiethylaminomethylethylene.

L14 ANSWER 20 OF 27 USPATFULL on STN

AN 2003:282653 USPATFULL

TI Minicell libraries

IN Surber, Mark W., Coronado, CA, UNITED STATES  
 Berkley, Neil, San Diego, CA, UNITED STATES  
 Gerhart, William, La Mesa, CA, UNITED STATES  
 Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003198996 A1 20031023

AI US 2002-157147 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2001-293566P 20010524 (60)  
 US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
 IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18482

AB The invention provides compositions and methods for the production of  
 achromosomal and anucleate cells useful for applications such as  
 diagnostic and therapeutic uses, as well as research tools and agents  
 for drug discovery.

DETD [0314] Regulatory elements, promoters and other expression elements and  
 expression factors from E. coli include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
 plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of

Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .  
 DETD . . . from prokaryotes other than E. coli and B. subtilis include  
 without limitation ahyRI gene product from Aeromonas hydrophila and  
 Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);  
 angR gene. . .  
 DETD . . . from prokaryotes other than E. coli and B. subtilis include  
 without limitation ahyRI gene product from Aeromonas hydrophila and  
 Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281);  
 angR gene. . .  
 DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
 traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
 bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
 membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene  
 (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
 from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic  
 Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J.  
 Jorgensen, and I. van. . . 8:2703-2709); the secE gene (Muller, M.,  
 et al. 1002. Protein traffic in bacteria: multiple routes from the  
 ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res.  
 Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002.  
 Protein traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol.  
 66:107-157).  
 DETD . . . that is in contact with the minicell composition. By  
 "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .  
 DETD [0472] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .  
 DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCdb), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .  
 DETD [0582] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access  
 through lipid membranes, which are generally hydrophobic. . .  
 DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
 also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
 blood-brain barrier to the brain. This modality is used, by way of  
 non-limiting example, for imaging purposes, and for. . .  
 DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino  
 acids such as polylysine, polyhistidine, polyornithine, polyquaternary  
 compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE),  
 DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran,  
 DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates,  
 polyamidoamines, cationic starches, polyvinylpyridine, and  
 polythiodiethylaminomethylethylene.

L14 ANSWER 21 OF 27 USPATFULL on STN  
 AN 2003:282652 USPATFULL  
 TI Forward screening with minicells  
 IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
 Berkley, Neil, San Diego, CA, UNITED STATES  
 Surber, Mark W., Coronado, CA, UNITED STATES  
 Gerhart, William, La Mesa, CA, UNITED STATES  
 PI US 2003198995 A1 20031023  
 AI US 2002-156831 A1 20020528 (10)  
 RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)  
US 2001-293566P 20010524 (60)  
DT Utility  
FS APPLICATION  
LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614  
CLMN Number of Claims: 15  
ECL Exemplary Claim: 1  
DRWN 2 Drawing Page(s)  
LN.CNT 18533

AB The invention provides compositions and methods for the production of  
achromosomal and anucleate cells useful for applications such as  
diagnostic and therapeutic uses, as well as research tools and agents  
for drug discovery.

DETD [0314] Regulatory elements, promoters and other expression elements and  
expression factors from *E. coli* include but are not limited to  
\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
traffic in bacteria: multiple routes from the ribosome to and  
\*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic  
Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
Jorgensen, and I. . . 8:2703-2709); the secE gene (Muller, M., et  
al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By  
"semipermeable" it is meant that certain agents can be freely exchanged  
\*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
the membrane or the other. For example, the toxic compound. . .

DETD [0471] Many Gram-negative pathogens use a type III secretion machine to  
translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
(for a review, see Cheng L W, Schneewind O. Type III machines of  
Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
domains (ABCdb), the majority of which energize the transport of  
compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
involved in the uptake of a wide range of molecules and in mechanisms  
of. . .

DETD [0580] A "channel protein" is a protein that facilitates the diffusion  
of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
hydrophilic pore or "channel" that provides molecules/ions access  
through lipid membranes, which are generally hydrophobic. . .

DETD [0669] XIII. Minicell-based Delivery of Biologically \*\*\*Active\*\*\*  
Agents

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can  
also be used for delivery of therapeutic agents \*\*\*across\*\*\* the  
blood-brain barrier to the brain. This modality is used, by way of

DET D non-limiting example, for imaging purposes, and for. . .  
. . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 22 OF 27 USPATFULL on STN

AN 2003:276773 USPATFULL

TI Minicell compositions and methods

IN Surber, Mark W., Coronado, CA, UNITED STATES

Sabbadini, Roger A., Lakeside, CA, UNITED STATES

PI US 2003194798 A1 20031016

AI US 2002-154951 A1 20020524 (10)

PRAI US 2001-293566P 20010524 (60)

US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 18

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18583

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DRWD [0345] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DRWD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DRWD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DRWD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DRWD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DRWD [0504] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope



(for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . . .

DRWD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . . .

DRWD [0613] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . . .

DRWD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . . .

DRWD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 23 OF 27 USPATFULL on STN

AN 2003:276689 USPATFULL

TI Minicell-based transformation

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Berkley, Neil, San Diego, CA, UNITED STATES  
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003194714 A1 20031016

AI US 2002-157299 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)  
US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18595

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0348] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to  
\*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals: Mol. Microbiol. 19:101-112);  
ampD (Lindquist, S., et al. 1989. Signalling proteins in. . . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5 S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene

(Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0505] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0613] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 24 OF 27 USPATFULL on STN

AN 2003:271080 USPATFULL

TI Minicell-based rational drug design

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Surber, Mark W., Coronado, CA, UNITED STATES

PI US 2003190683 A1 20031009

AI US 2002-157302 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)  
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 15

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18539

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate .

N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . . .

DETD . . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and l.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . . .

DETD [0503] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . . .

DETD . . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . . .

DETD [0612] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . . .

DETD . . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . . .

DETD . . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\* , DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolyethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 25 OF 27 USPATFULL on STN

AN 2003:270998 USPATFULL

TI Target display on minicells

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
Berkley, Neil, San Diego, CA, UNITED STATES  
Surber, Mark W., Coronada, CA, UNITED STATES

PI US 2003190601 A1 20031009

AI US 2002-157096 A1 20020528 (10)

RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING

PRAI US 2002-359843P 20020225 (60)  
US 2001-293566P 20010524 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18581

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0349] Regulatory elements, promoters and other expression elements and expression factors from *E. coli* include but are not limited to \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of *Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112); ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include without limitation ahyRI gene product from *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and I. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0504] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0609] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyomithine, polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copoly methacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 26 OF 27 USPATFULL on STN

AN 2003:238122 USPATFULL

TI Minicell-based transfection  
 IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
 Berkley, Neil, San Diego, CA, UNITED STATES  
 PI US 2003166279 A1 20030904  
 AI US 2002-157391 A1 20020528 (10)  
 RLI Division of Ser. No. US 2002-154951, filed on 24 May 2002, PENDING  
 PRAI US 2002-359843P 20020225 (60)  
 US 2001-293566P 20010524 (60)  
 DT Utility  
 FS APPLICATION  
 LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR,  
 IRVINE, CA, 92614  
 CLMN Number of Claims: 18  
 ECL Exemplary Claim: 1  
 DRWN 2 Drawing Page(s)  
 LN.CNT 18548  
 AB The invention provides compositions and methods for the production of  
 achromosomal and anucleate cells useful for applications such as  
 diagnostic and therapeutic uses, as well as research tools and agents  
 for drug discovery.  
 DETD [0312] Regulatory elements, promoters and other expression elements and  
 expression factors from *E. coli* include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\*  
 plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of  
*Escherichia coli* by global stress signals. *Mol. Microbiol.* 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . from prokaryotes other than *E. coli* and *B. subtilis* include  
 without limitation ahyRI gene product from *Aeromonas hydrophila* and  
*Aeromonas* \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing  
 in *Aeromonas hydrophila* and *Aeromonas* \*\*\*salmonicida\*\*\* :  
 identification of the LuxRI homologs AhyRI and AsaRI and their cognate  
 N-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179:5271-5281);  
 angR gene. . .  
 DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein  
 traffic in bacteria: multiple routes from the ribosome to and  
 \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in  
 bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the  
 membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157); the FtsY gene  
 (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes  
 from the ribosome to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic  
 Acid Res. Mol. Biol.* 66:107-157); the fimC gene (Klemm P., and B. J.  
 Jorgensen, and l.. . . 8:2703-2709); the secE gene (Muller, M., et  
 al. 1002. Protein traffic in bacteria: multiple routes from the ribosome  
 to and \*\*\*across\*\*\* the membrane. *Prog. Nucleic Acid Res. Mol. Biol.*  
 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic  
 in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*  
 the membrane. *Prog. Nucleic Acid Res. Mol. Biol.* 66:107-157).  
 DETD . . . that is in contact with the minicell composition. By  
 "semipermeable" it is meant that certain agents can be freely exchanged  
 \*\*\*across\*\*\* the membrane, whereas others are retained on one side of  
 the membrane or the other. For example, the toxic compound. . .  
 DETD [0469] Many Gram-negative pathogens use a type III secretion machine to  
 translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope  
 (for a review, see Cheng L W, Schneewind O. Type III machines of  
 Gram-negative bacteria: delivering. . .  
 DETD . . . a database devoted to the ATP-binding cassette (ABC) protein  
 domains (ABCD), the majority of which energize the transport of  
 compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are  
 involved in the uptake of a wide range of molecules and in mechanisms  
 of. . .  
 DETD [0578] A "channel protein" is a protein that facilitates the diffusion  
 of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a  
 hydrophilic pore or "channel" that provides molecules/ions access

through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.

L14 ANSWER 27 OF 27 USPATFULL on STN

AN 2003:237942 USPATFULL

TI Minicells comprising membrane proteins

IN Sabbadini, Roger A., Lakeside, CA, UNITED STATES  
 Surber, Mark W., Coronado, CA, UNITED STATES  
 Berkley, Neil, San Diego, CA, UNITED STATES  
 Segall, Anca M., San Diego, CA, UNITED STATES  
 Klepper, Robert, San Diego, CA, UNITED STATES

PI US 2003166099 A1 20030904

AI US 2002-157305 A1 20020528 (10)

PRAI US 2001-295566P 20010605 (60)  
 US 2002-359843P 20020225 (60)

DT Utility

FS APPLICATION

LREP KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614

CLMN Number of Claims: 20

ECL Exemplary Claim: 1

DRWN 2 Drawing Page(s)

LN.CNT 18580

AB The invention provides compositions and methods for the production of achromosomal and anucleate cells useful for applications such as diagnostic and therapeutic uses, as well as research tools and agents for drug discovery.

DETD [0346] Regulatory elements, promoters and other expression elements and expression factors from E. coli include but are not limited to  
 \*\*\*acrR\*\*\* (Ma, D., et al. 1996. The local repressor \*\*\*AcrR\*\*\* plays a modulating role in the regulation of \*\*\*acrAB\*\*\* genes of Escherichia coli by global stress signals. Mol. Microbiol. 19:101-112);  
 ampD (Lindquist, S., et al. 1989. Signalling proteins in. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . from prokaryotes other than E. coli and B. subtilis include without limitation ahyRI gene product from Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* (Swift, S., et al. 1997. Quorum sensing in Aeromonas hydrophila and Aeromonas \*\*\*salmonicida\*\*\* : identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J. Bacteriol. 179:5271-5281); angR gene. . .

DETD . . . 175:5009-5021); the ffh gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); 4.5S RNA (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the FtsY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); the fimC gene (Klemm P., and B. J. Jorgensen, and 1.. . . 8:2703-2709); the secE gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\* the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157); and the secY gene (Muller, M., et al. 1002. Protein traffic in bacteria: multiple routes from the ribosome to and \*\*\*across\*\*\*

the membrane. Prog. Nucleic Acid Res. Mol. Biol. 66:107-157).

DETD . . . that is in contact with the minicell composition. By "semipermeable" it is meant that certain agents can be freely exchanged \*\*\*across\*\*\* the membrane, whereas others are retained on one side of the membrane or the other. For example, the toxic compound. . .

DETD [0502] Many Gram-negative pathogens use a type III secretion machine to translocate protein toxins \*\*\*across\*\*\* the bacterial cell envelope (for a review, see Cheng L W, Schneewind O. Type III machines of Gram-negative bacteria: delivering. . .

DETD . . . a database devoted to the ATP-binding cassette (ABC) protein domains (ABCdb), the majority of which energize the transport of compounds \*\*\*across\*\*\* membranes. In bacteria, ABC transporters are involved in the uptake of a wide range of molecules and in mechanisms of. . .

DETD [0611] A "channel protein" is a protein that facilitates the diffusion of molecules/ions \*\*\*across\*\*\* lipid membranes by forming a hydrophilic pore or "channel" that provides molecules/ions access through lipid membranes, which are generally hydrophobic.. . .

DETD . . . sweat, saliva or a tissue such as liver or heart. Minicells can also be used for delivery of therapeutic agents \*\*\*across\*\*\* the blood-brain barrier to the brain. This modality is used, by way of non-limiting example, for imaging purposes, and for. . .

DETD . . . polymers include acidic gelatin, polygalactosamine, polyamino acids such as polylysine, polyhistidine, polyornithine" polyquaternary compounds, prolamine, polyimine, diethylaminoethyl dextran (DEAE), DEAE-imine, DEAE-methacrylate, DEAE- \*\*\*acrylamide\*\*\*, DEAE-dextran, DEAE-cellulose, poly-p-aminostyrene, polyoxethane, copolymethacrylates, polyamidoamines, cationic starches, polyvinylpyridine, and polythiodiethylaminomethylethylene.